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Communicative interaction of myosins along an actin filament in the presence of ATP

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Abstract

Myosin molecules contacting an actin filament in the presence of ATP were found to regulate the filamental fluctuations due to ATP hydrolysis in a communicative manner along the filament. As an evidence of the occurrence of the communication, ATP-activated fluctuating displacements of the filament in the direction perpendicular to its longitudinal axis were identified to propagate at a finite velocity not less than about 0.2 μm/s unidirectionally along the filament.

Keywords: Actin; ATP; Filamental fluctuations; Myosin

1. Introduction

Sliding movement of the actomyosin complex and its ATP hydrolysis, which underlie muscle contraction, are a major subject of how physics works in biology. One attempt in this direction is to see how physically measurable quantities that are available in actomyosin complexes develop in time [1–6]. In the preceding report [7], we measured and characterized the intensity of the fluctuating displacements of an actin filament in the direction perpendicular to its longitudinal axis when it interacts with myosin in the presence of ATP. What has been significant to these transversal fluctuations is the possibility that the contact regions of actins and myosin distributed over the actin filament could be coordinated among themselves. For the kinetic energy of transversal fluctuations has been suggested that it might be transformed into the uniform sliding movement of the filament.

In the present article we report that ATP-activated transversal fluctuations of an actin filament were unidirectionally propagative along the filament, implying that myosin molecules distributed over the filament regulate their timing of ATP-activated displacements mutually in a communicative manner.

2. Materials and methods

We followed completely the similar materials and methods already reported in Hatori et al. [7]. The only new additions are on the ATPase activity and on how transversal fluctuations of an actin filament propagates along the filament. The measurement of ATPase activity was done by following the standard procedure using reactions with pyruvate kinase and lactic dehydrogenase [8]. The intensity of the

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transversal fluctuations was measured as the displacement of a point on the skeleton-line of an actin filament in the direction perpendicular to the filament. The measured displacements were averaged both spatially over 40 points (93 nm apart, equally) on the same filament and temporally over 8 s.

3. Results

3.1. ATPase activity

The ATPase activity of an actin filament at a myosin concentration of 100 μg/ml, which was kept throughout our entire experiment, was measured at room temperature (see Fig. 1). For comparison, the results of the transversal fluctuations of an actin filament measured under the similar conditions are reproduced in the figure, in which fluctuations are represented in terms of the standard deviation of the transversal displacements. An almost similar ATP-dependence can be observed for both the transversal fluctuations of an actin filament and the ATPase activity.

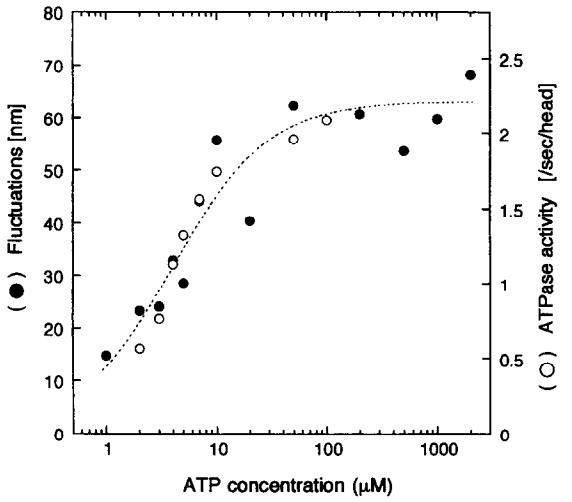

Fig. 1. ATPase activity vs. ATP concentration. The ATPase activity measured as the number of ATP molecules hydrolyzed per second at each myosin head (open circle) is contrasted against the intensity of the fluctuating transversal displacements of an actin filament measured in their standard deviation (filled circle), the latter of which was reproduced from our previous work [7].

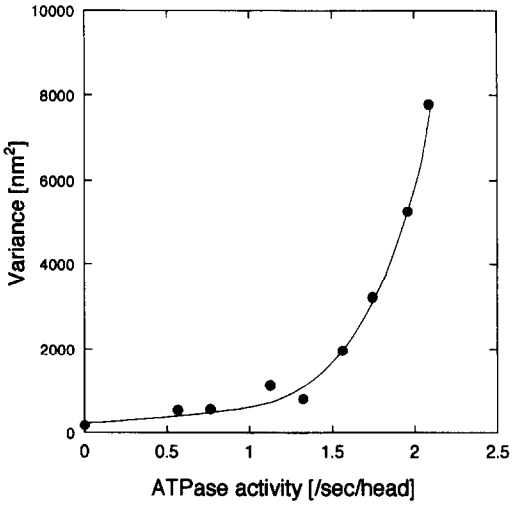

Fig. 2. Power intensity of the transversal fluctuations of an actin filament measured in variance of the displacements vs. the ATPase activity.

3.2. ATPase activity and fluctuation power

The relationship between the ATPase activity and the fluctuation power of an actin filament at an arbitrary point on the skeleton-line measured in square or variance of the perpendicular displacements averaged over time is demonstrated in Fig. 2. As the ATPase activity went beyond a certain threshold, the fluctuation power was found to increase significantly with the activity until both reached the plateau.

3.3. Propagative fluctuations

In order to examine whether or not the transversal fluctuations would propagate along the actin filament, we measured the cross correlation of the fluctuation intensities at two separate points on the skeleton-line of the filament, while choosing the time difference between these fluctuating variables as a parameter. Details of the method have already been reported in Honda et al. [9]. The results of the cross correlation at 1 μM ATP concentration are presented in Fig. 3. The maximum point of the cross correlation was found to move into exactly the same direction as the actin filament slid into when ATP-activated. That is to say, the propagative fluctuations

turned out to be unidirectional. The transversal fluctuations were also observed to vary their propagation velocity with the increase of ATP concentration (see

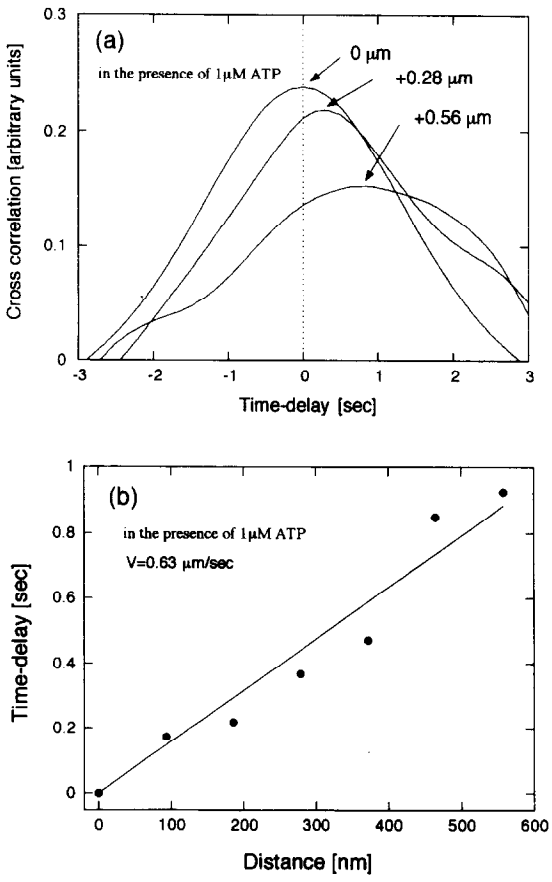

Fig. 3. (a) Cross correlation function of the transversal displacements at two separate points along the longitudinal axis parameterized in terms of the time-delay between the two. The distance marked in the figure denotes the separation over which the cross correlation of displacements was estimated. The time denoted along the abscissa measures the time-delay for evaluating the cross correlation. The positive sign in front of each designated distance in the figure implies that the time-delay is counted positive when the delay is taken to occur in the direction in parallel to which the filament slides, and negative otherwise. The displayed cross correlation is an ensemble average of temporal averages over 8 s, taken over 10 independent sample points along the filament. (b) Distance between two separate points along the actin filament vs. the time-delay that maximizes the cross correlation of transversal displacements over the designated distance. The apparent linearity between the distance and the time-delay determines the propagation velocity of the fluctuations.

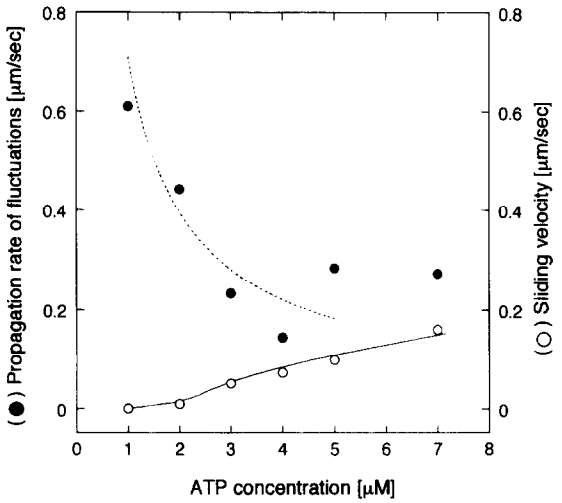

Fig. 4. Propagation velocity or rate of transversal fluctuations vs. ATP concentration. For comparison, the sliding velocity of the actin filament is also presented (reproduced from [7]).

Fig. 4). For comparison, the sliding velocity of the filament is also reproduced there.

4. Discussion

In view of the fact that the intensity of propagative fluctuations along the actin filament increased with the increase of the concentration of ATP to be hydrolyzed, what was propagated along the filament, turns out to be a signal for timing the filamental displacement due to ATP hydrolysis at each myosin in the line. The propagation velocity of the order of or slightly less than 1 μm/s reflects an extreme slowness of the turnover rate of ATP at each individual site of myosin along the filament [10]. Of course, the intervening regions between adjacent myosins on the filament can be communicated by various molecular vibrations whose propagation velocity may be more than several orders of magnitude greater than 1 μm/s [10,11]. But, most of the time required for signal-processing for ATP-activated filamental displacements would be spent in each localized region of the ATP-activated actomyosin complex placed in series along the filament.

One possible factor for determining the propagation velocity of those ATP-activated fluctuations may

be the number of ATP-active myosins over a unit length of the actin filament. In fact, the propagation velocity of fluctuations decreased with the increase of the ATP concentration we examined. As the number of intervening ATP-active myosins increases along the filament, the propagation velocity of a signal for timing the ATP-activated filamental displacements would decrease.

What is more, those ATP-activated propagative fluctuations were unidirectional. They moved in exactly the same direction as the filament slid into. The present unidirectional characteristic of propagative fluctuations can provide a further clue to what would actually be transferred and communicated in the propagation process.

Unidirectional propagation of a signal for timing the filamental displacement due to ATP hydrolysis is to communicate it asymmetrically in time along the actin filament [12]. It takes some time for a myosin molecule in the downstream along the filament to start regulating the ATP-activated fluctuations as responding to the similar regulation in the upstream. As a matter of fact, any communication that is temporally asymmetric is to exert a certain form of action upon any object to be communicated, while the reaction from the object is not concurrent with the action because of the temporal asymmetry of the communication. No communication presumes concurrency of action and reaction [13–15]. Nonetheless, the counterbalance of action and reaction has to be established at any rate, otherwise the third law of mechanics would be violated [13].

Communication resulting in the counterbalance of action and reaction in the absence of concurrent synchronization between the two has to be at best through asynchronous updating among the concerned parties [12,16]. The synchronous counterbalance of action and reaction is in accordance with an well-established one-to-one temporal mapping as in classical mechanics in the sense that both are determined uniquely at every moment, while the asynchronous one yields a motion of a one-to-many mapping since no action determines or anticipates its reaction uniquely in advance [13].

Establishing the counterbalance of action and reaction, that is common to any communication systems, is through either synchronous or asynchronous updating. Although physics in general and mechanics in particular has paid much attention to the possibility of globally concurrent synchronization of action and reaction as with the actomyosin complex in the absence of ATP, those material phenomena in biology as with the actomyosin complex in the presence of ATP witness actualization of a locally asynchronous updating of action and reaction for their counterbalance.

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