Movement Regulation of a Sliding Actin Filament in a Reconstruction Motility Assay System

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Abstract. An actomyosin system, which is the mechano-chemical systems of proteins, in a reconstruction motility assay system is one of many effective systems for discussing the formation mechanism of self-organized orders at protein levels. In this study we investigated the transformation change of sliding actin filaments using the optical microscopy technique and image analysis. As a result, the local windings propagated along an actin filament, and complex patterns of windings were formed. The three conditions of winding propagations need the following; the intermittent actions from myosins to the actin filaments, the connections between actin monomers have nonlinearity, and the actin filaments are flexible. These results suggest that the actin filaments have the regulation mechanism, which propagate the actions of myosins as windings.

1. Introduction

In biology, many kinds of complex behaviors such as periodic oscillations, or bursting, are observed at various temporal-spatial levels [1]. These biological orders are understood as one of the dissipative structures in non-equilibrium open system, which exchanged the substances, energy and information with environment. The dissipative structures depends on (i) specific nonlinear dynamics taking place in subsystems, and (ii) the way the subsystems couple. The biological system and non-biological systems, such as BZ reaction or crystal growth, have a common formative mechanism of dissipative structures. It is necessary to clarify the formation mechanism of living organisms at various temporal-spatial levels for understanding the biological machine.

Biological motions such as muscle contraction and flagellar movement, or nerve systems are the systems of living organisms, which show the complex and various behaviors. These systems have the mechano-chemical systems of proteins, and play an important function in biology. Therefore clarifying the self-organization mechanism of those systems is one of the clues for understanding living organisms.

Murase et al. investigated the self-organization phenomena on flagellar movement, which has a dynein-tubulin system as a mechano-chemical system [1]. They showed that the difference of time constants between the activated state and the inactivated state of dynein and the structural anisotropy of dynein are factors of the self-organized mechanism of movement. This result suggests that the structural characteristics or time response of proteins are important factors for complex behaviors in mechano-chemical systems of proteins.
An actomyosin system, which plays a role in muscle contractions, is also one of the mechano-chemical systems. The sliding movement of the actin filaments, which is the molecular mechanism of muscle contractions, has been studied. This sliding mechanism of the actin filament is generally explained using the Lever Arm Model, or the Loose Coupling Model. In such models, the mechanism of sliding movement was explained that the actin filaments moved by conformation changes of myosins using the energy of ATP hydrolysis. However, for details of relaxation process in conformational distortion of myosins, it is not clear whether myosins move actin filament directly, or the actin filaments move after conformation distortion of myosins is transformed into the conformation distortion of actin filaments.

In this study we discuss that the actin filaments regulate the actions of myosins by the flexion movement of the sliding actin filament. We clarify the factors of the self-organized mechanism of the flexion movement in sliding movement of actin filament. We suggest that the structural characteristics or time response of the actin filaments, which is a protein, are factors of nonlinearity in subsystems.

2. Materials and Methods

2.1. Reagents and proteins
Actin and Myosin were prepared from rabbit skeletal muscle [8]. Heavy mero-myosin (HMM) was prepared by α-chymotryptic digestion of myosin [9]. Actins were extracted from acetone powder and were purified according to the method of Spudich and Watt [10]. Fluorescent-labeled actin filaments were made and treated with equal molar rhodamin-phalloidin (Sigma Co.) in buffer A (25 mM imidazole-HCl, 25 mM KCl, 4 mM MgCl2, 1 mM DTT, pH=7.4).

2.2. A reconstruction motility assay system and observations of actin filaments
We prepared a standardized reconstruction motility assay system (Figure 1.)[7]. HMMs dissolved in buffer A were perfused between a cover glass (18x18 mm) and a slide glass (24x36 mm) coated with collodion (Sigma Co.). Roughly 60 sec after the perfusion, HMMs that did not attach on the glass surface were removed with a bovine serum albumin dissolved in buffer A. Fluorescent-labeled actin filaments dissolved in buffer A were then perfused, and put on HMMs. ATP dissolved in buffer A was then perfused, and the sliding movements of actin filaments were started.

The observation of fluorescent-labeled actin filaments was performed under an optical microscope with a fluorescent illumination unit (Olympus, IX70, X100 oil objective lens, X1.5 and X2.0 relay lens). The fluorescent images were recorded on hard disk by using a personal computer with a high sensitive video camera (Hamamatsu Photonics, C2400-97V) at intervals of 1/30 sec.

2.3. Image analysis
The skeletons of actin filaments in fluorescent images were analyzed using a thinning program, written in C language with Visual Studio 2005 (Microsoft Co.), as shown in Figure 2 and Figure 3. Three consecutive data of the skeleton data of an actin filament were averaged. Here, the head-end of the filaments was defined as the end of the direction of movement, and the tail-end of the filaments was defined as the end of the backward direction of movement. The winding angle of some coordinate point \( P_j(x,y) \) along the skeleton of action filament is estimated from the following formula.

\[
\theta_j = \left( \frac{180}{\pi} \right) \cos^{-1} \left( \frac{\left( P_{j+1}(x,y) - P_{j+1}(x,y) \right) \cdot \left( P_j(x,y) - P_j(x,y) \right)}{\left\| P_{j+1}(x,y) - P_j(x,y) \right\| \left\| P_j(x,y) - P_j(x,y) \right\|} \right)
\]
3. Results

3.1. Winding propagation along an actin filament

Figure 4 shows the time developments of displacement of the head-end and tail-end, and the variety of winding angles of an actin filament at every 1/30 sec. Each graph in Figure 4 shows the well known tendency that the sliding velocity increased as ATP concentration increased. The sliding velocity of actin filaments is about 1 μm/sec at low concentration of 0.02 mM ATP, as shown in Figure 4(a), and 4~5 μm/sec at high concentration of 2 mM ATP, as shown in Figure 4(b). On the other hand, the time developments of each winding angle at the head-end and at the tail-end of an actin filament decreased as ATP concentration increased. Actin filaments showed slow sliding movement with frequent change in direction at low concentration of 0.02 mM ATP. Actin filaments also showed straight and smooth movement at high concentration of 2 mM ATP.

Moreover, at each concentration of ATP the change of winding angle at the tail-end was clearly large in comparison with those at the head-end. Additionally, the difference between the head-end and the tail-end tended to increase as ATP concentration increased. In other words, the number of windings along the actin filaments increased as the sliding velocity increased, but the tail-end part of the actin filament had the windings while the head-end part of the actin filament became straight.

Figure 5 shows the time development of winding angle on the skeleton of an actin filament by kymograph. The horizontal axis is time. The vertical axis is the distance from the head-end of the actin filament.
filament to a given value of the actin filament. The pixelated grayscale concentration displays the winding angle. The diagonal pattern was formed by the assembly of the high concentration pixels. It shows the second-order propagation of windings along an actin filament. Its gradient is the propagation velocity. Moving diagonally in a right upward direction displays the propagation from the head-end to the tail-end. Conversely, the right downward direction displays the propagation from the tail-end to the head-end. The area filled with light colored pixels shows the domain on a sliding actin filament in which the winding angle does not change. Generally, this domain is almost straight.

From these figures, we could see the generation of complex winding patterns along an actin filament in sliding movement. The difference of these patterns depended on ATP concentration. The fastest propagation velocity was about twice the ordinal average velocity at which the actin filaments slide at each concentration of ATP. The large windings tended to propagate in a backward direction in a high velocity sliding movement at the high concentration of ATP.

On the other hand, we found a complex phenomenon caused by changing the winding mode. For example, in Figure 5 (b), the winding propagation of the velocity of $5 \sim 10 \, \mu \text{m/sec}$ from the tail-end to the head-end reflected at the head-end point. The windings also propagated from the head-end to the tail-end at the velocity of $5 \sim 10 \, \mu \text{m/sec}$ at 0.5-2.0 sec. In Figure 5 (b), the propagation from the tail-end to the head-end switched to the propagation from the head-end to the tail-end at 1.5 sec after starting. Here, some of the winding propagations assembled after the direction of winding propagation switched their direction. After that, the whole filament become almost straight and continued its sliding movement.

![Figure 4](image1.png)  
**Figure 4.** Time series of displacement and change of winding angle on the head-end and the tail-end of the sliding actin filament.

![Figure 5](image2.png)  
**Figure 5.** Kymographs of winding propagation along an actin filament.
3.2. Winding propagation on a one-dimensional elastic body model

The windings of actin filaments occurred on both the non-sliding actin filaments and the sliding actin filaments. However, the winding propagation along an actin filament only occurred on the sliding actin filaments. This result shows that the occurrence of winding propagations needs the process in which the myosins dissociate from actin filaments in the presence of ATP. In other words, the actin filaments need the actions of activated-myosins for winding propagations. The winding propagation patterns depended on the sliding velocity of actin filaments. This result shows that the winding propagation occurs not only due to the actions of myosins, but also to the internal actions of actin filaments. Therefore, we investigated the propagation process along the local windings of actin filaments using an one-dimensional elastic body model.

\[
\frac{d^2 \theta_j}{dt^2} = (M_{j+1} - M_j) + n \cdot \sin \theta_j
\]

\[
M_j = K_l (\theta_j - \theta_{j+1}) + K_n (\theta_j - \theta_{j+1})^3
\]

Here, \( \theta \) is the winding angle between units, \( M_j \) is the restoring moment of the connection springs, \( n \) is the switch of external action, \( K_l \) and \( K_n \) are the spring constants, and \( j \) is a data number of one-dimensional elastic body. This one-dimensional elastic body model is dissolved by computer simulation using the Runge-Kutta method. As a result, similar local windings in the sliding actin filaments were found when the model meets the following three conditions. Firstly, the external actions to the subsystem are intermittent. Secondly, the connection springs has nonlinearity. Finally, the spring constant is small.

4. Discussion

The mechanism of sliding movement of actin filament is generally explained in the models, which are based on the myosins with the ATP hydrolysis activities. In those models, the actin filaments play the role of rail, which are moved by myosins. On the other hand, Hatori and Honda et al. showed that the propagation of transversal fluctuations and acceleration along the actin filaments occur while sliding [7][11]. These results suggest that the myosin-based model is not enough to explain the movement of actin filaments. Therefore, another model is needed for the sliding movement of actin filaments.

In this study, we investigate the mechanism which regulates the actions from actin filaments to myosins. As a result, the local windings of actin filaments formed the complex patterns, and propagate along actin filaments. The propagation patterns depended on the sliding velocity or ATP concentration. The fastest propagation velocity of windings along a sliding actin filament was about twice the ordinal average velocity at which the actin filaments slide at each concentration of ATP. In addition, the windings propagate both from the head-end to the tail-end and from the tail-end to the head-end. These results show that the windings of actin filaments are not passive. In fact, these results suggest the possibility that the winding propagation of actin filaments contribute to the mechanism of sliding movement though actin filaments do not have the ATP hydrolysis activities.

We simulated the winding propagation along actin filaments by a one-dimensional elastic body model. The winding propagation along a one-dimensional elastic body was found when the model meets the three conditions stated above. Firstly, the external actions to the subsystems are intermittent. This corresponds to the intermittent actions between the actin filaments and myosins. In fact, the actin filaments associate the myosins intermittently [12]. Secondly, the connection springs has nonlinearity. This means that the actin monomers have structural anisotropy. Finally, the spring constant is small. This spring constant corresponds to the binding strength between actin monomers. The actin monomers in the actin filaments are connected with electrostatic action. As a result, the actin filaments have flexibility. These results show that the factors of winding propagation along actin filaments are the intermittent actions between the actin filaments and myosins, the nonlinearity of actin monomers, and the weak connection between actin monomers in the actin filaments.

This action between actin filaments and myosins occurs within milliseconds. On the other hand, the winding propagation of actin filaments occurs within seconds. In fact, the actions of winding propagation...
along actin filaments have two different time scales. This property has a resemblance to the property of other various and complex behaviours, such as flagellar movement. These results suggest that the actin filaments have the regulation mechanism, which propagate the actions of myosins as windings to the neighboring region.

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