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Contractile and protractile coordination within an actin filament sliding on myosin molecules

Hajime Honda[a], Kuniyuki Hatori[a], Yoshimasa Igarashi[a], Katsuhiko Shimada[b], Koichiro Matsuno[a,*]

[a] *Department of BioEngineering, Nagaoka University of Technology, Nagaoka 940-2188, Japan*
[b] *School of Design and Architecture, Nagoya City University, Nagoya 464, Japan*

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Abstract

An actin filament exhibits distortions longitudinally when it slides upon myosin molecules. We observed that the actin filament demonstrated contractile distortions at low ATP concentrations and protractile distortions at high concentrations. Temporal development of such distortions was identified, by tracing each of several speckled fluorescent markers attached to the actin filament. Close association of the sliding movement to the moving distortions of an actin filament suggests the presence of a unitary mechanism regulating the apparently two different modes of dynamic movement.

Keywords: Actin filament; Contraction; Distortion; Myosin; Protraction

1. Introduction

An actin filament sliding on myosin molecules in the presence of ATP to be hydrolyzed presumes at least two different processes; one is for how the energy stored in ATP could be transformed into the mechanical energy for actualizing the sliding movement [1–8], and the other is for how each actomyosin complex along the filament could come to start hydrolyzing an ATP molecule in relation to the similar ones in the immediate neighborhood [9–14]. If the neighboring actomyosin complexes on the actin filament are totally disorganized among themselves with regard to their initiations of ATP hydrolysis, a considerable amount of mechanical stains or distortions along the filament could arise [15,16]. The absence of a coordination of ATP hydrolyses along the filament could yield the push–pull distortions longitudinally due to the high likelihood of letting each actomyosin complex on the filament be the

* Corresponding author.

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mechanical load to the similar ones in the immediate neighborhood. However, occurrence of the higher mechanical distortions is unstable for the perturbations lowering them. Further explication of those actomyosin complexes urges us to address the question of what could be responsible for lowering the possible mechanical distortions to be generated in an actin filament sliding on myosin molecules.

In the present article, we shall examine the nature of mechanical distortions to appear in the actin filament sliding on myosin molecules and the dynamic scheme of how these distortions could be mitigated. For this purpose, we prepared a speckled actin filament marked by fluorescent materials [16]. Such a speckled actin filament enables us to estimate how the filament could be distorted longitudinally during its sliding movement on myosin molecules.

2. Materials and methods

2.1. How to prepare a speckled actin filament? [16]

Actin and myosin molecules were prepared from rabbit skeletal muscle [17–19]. Speckled actin filaments were made from a mixture of actin filaments both unlabeled and labeled by rhodamine-phalloidine. 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 (Yamato, Branson 3200) for 4 min. The mixture of dissected actin filaments, both labeled and unlabeled mixed together, was placed at 4°C for 24 h for their reformation of the filaments that could be speckled ones.

2.2. Measurement apparatus

We prepared a standardized in vitro motility assay [20]. The slide glass to fix HMM of concentration 0.2 mg/ml was hydrophobically treated with butyltrimethoxysilane (Shinetsu Chem. Co.). 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 $MgCl_2$, 20 μM or 2 mM ATP and 1 mM DTT at a temperature of 25°C. The microscope (Olympus, IX70) attached with the object lens (Olympus, Uplan Apo 100×, oil) was used with the aid of the fluorescent equipment (Olympus, IX-FLA) and the fluorescent filter (Olympus, rhodamine filter). The image of the microscope was taken by a video cassette recorder (Sony, EVO-9500A) carrying the ICCD-Camera (Video Scope International, Ltd, ICCD-350F). The images were processed by the image-processing software; NIH Image 1.6 (Wayne Rasband, National Institute of Health, Research Services). Each image was taken at every 1/30 s through video grabber board (Scion Co., LG-3 PCI) into a computer. The spacing of neighboring pixels was 100 nm, and the spatial resolution of identifying the end of each speckled segment along the filament was 100 nm. Observation of the displacement of an actin filament was done at every interval of 1/30 s.

2.3. 'Madara' approximation

Further examination of the dynamic nature of a speckled actin filament sliding on myosin molecules will be attempted by referring to the skeleton length of the actin filament (see Fig. 1), which has been renamed as the filamental length in the *madara* approximation by Koshin Mihashi[1]. The skeleton length, L, is the total sum of the length of each linear segment sandwiched by two adjacent fluorescent speckles. Another numerical figure of interest will be the filamental winding index, W, measuring the ratio of the end-to-end distance, D, to the skeleton length, L, of the actin filament. The winding index W will tell us to what extent the actin filament would be winding during its sliding movement. If the index W approaches unity from below, this would imply that the filament would come to be straight as de-

[1]The term *madara* is a Japanese synonym of speckled pattern in general.

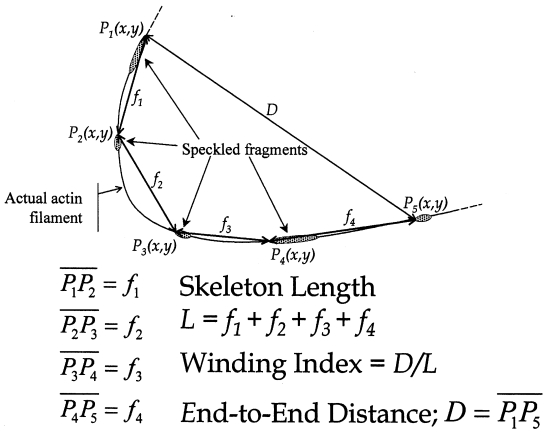

Fig. 1. A schematic representation of the *madara* approximation. Skeleton length, L, measures the length of a speckled actin filament as referring to each front portion of several speckled markers, while winding index, W, measures the ratio of the end-to-end distance, D, to skeleton length, L.

creasing the extent of winding. In particular, the case of large L and large W corresponds to a protractile distortion, and the case of small L and large W to a contractile one. Based upon this classification scheme, we shall examine the development of these variables at both low and high ATP concentrations.

3. Results and discussion

The results of the development of the skeleton length and the winding index are presented in Fig. 2, in which the ATP concentration was chosen at 20 μM. A significant feature is that the winding index approached unity while the skeleton length became minimized. This implies that the actin filament exhibited contractile distortions. The extent of contractile distortions was as much as 10% of the total length of the filament. We did not identify any apparent evidence of buckling distortions [21,22] under the condition of the present observation under a microscope. In contrast, when the ATP concentration was as high as 2 mM (see Fig. 3), there was no explicit indication of such contractile distortions. However, even if mechanical distortion survives there, it must be of a protractile character compared to the contractile ones with a low (20 μM) ATP concentration. In either case of low and high ATP concentrations, we could not observe any explicit correlation of fluctuations of the sliding velocity between the front and the end segment of the actin filament. This implies that those distortions, whether contractile or protractile, could be correlated only locally within the actin filament.

Our observation of the contractile distortions of an actin filament at low ATP concentrations must be related to some internal deformation of the actin monomers in the filament. X-Ray diffraction studies of thin actin filaments reveal that each actin subunit exhibits its stretching as much as approximately 0.3% during isometric contraction of muscles at full overlap length of thick and thin filaments [23–26]. Although deformations of each actin monomer constituting the actin filament would certainly be involved in our measurement, the contractile distortions as much as of 10% in the total length of an actin filament could not be attributed to such deformations of each actin subunit alone. A likely candidate for such sizable contractile distortions may be due to transversal displacements of the actin filament as depicted in Fig. 4. In fact, our previous studies [15] demonstrated an occurrence of the transversal displacements of an actin filament at extremely low ATP concentrations that propagated in the forward direction.

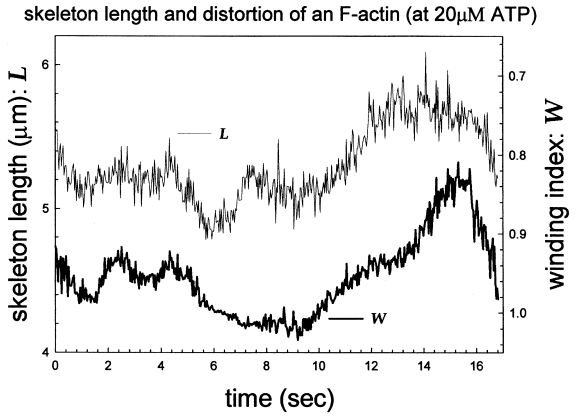

Fig. 2. Time development of both skeleton length, L, of a speckled actin filament and winding index, W, at 20 μM ATP concentration.

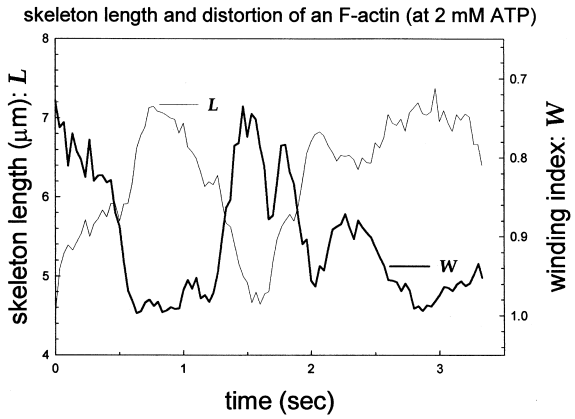

Fig. 3. Time development of both skeleton length, L, of a speckled actin filament and winding index, W, at 2 mM ATP concentration.

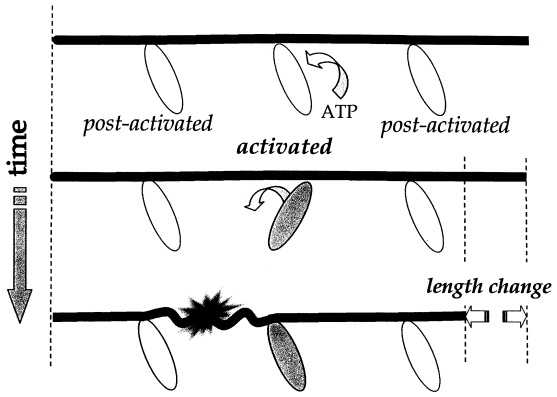

Fig. 4. A likely mechanism underlying a development of contractile distortions of an actin filament at low ATP concentrations, in which it is noted that those myosin molecules actively involved in ATP hydrolyses concurrently along a single actin filament are relatively sparse.

On the other hand, as the ATP concentration increases, the mechanical distortions expected to occur along the filament could become protractile in the sense of decreasing the extent of contractile distortions. Protractile distortions in the form of variations in the longitudinal acceleration at the ATP concentration as high as 2 mM were observed to propagate in the backward direction [16].

4. Concluding remarks

Mechanical distortions occurring in the actin filament while contacting myosin molecules in the presence of ATP must be mitigated. Otherwise, those distortions would make the filament mechanically unstable [27,28]. The manner in which those mechanical distortions are mitigated is at the least through either ATP hydrolyses or not. Our present observation of mechanical distortions in the actin filament in the presence of ATP reveals an indefinite sustenance of mechanical distortions, whether they are contractile or protractile ones. Surviving distortions manifest further generations of mechanical distortions as responding to the process of mitigating the preceding ones in a propagative manner along the actin filament. This may suggest that mechanical distortion, whether contractile or protractile, could serve as a factor coordinating mechanochemical couplings in actomyosin complexes along an actin filament.

References

[1] H.E. Huxley, Science 164 (1969) 1356–1366.
[2] A.F. Huxley, R.M. Simmons, Nature 233 (1971) 533–538.
[3] Y. Suzuki, T. Yasunaga, R. Ohkura, T. Wakabayashi, K. Sutoh, Nature 296 (1998) 380–383.
[4] I. Dobbie, M. Linari, G. Piazzesi, M. Reconditi, N. Koubassova, V. Lombardi, M. Irving, Nature 396 (1998) 383–387.
[5] J.T. Finer, R.M. Simmons, J.A. Spudich, Nature 368 (1994) 112–119.
[6] A. Ishijima, H. Kojima, T. Funatsu, M. Tokunaga, H. Higuchi, H. Tanaka, T. Yanagida, Cell 92 (1998) 161–171.
[7] F. Oosawa, J. Biochem. (Tokyo) 118 (1995) 863–870.
[8] S. Higashi-Fujime, T. Hozumi, Biochem. Biophys. Res. Commun. 221 (1996) 267–272.
[9] K. Hatori, H. Honda, K. Matsuno, Biophys. Chem. 58 (1996) 267–272.
[10] K. Hatori, H. Honda, K. Shimada, K. Matsuno, Biophys. Chem. 70 (1998) 241–245.
[11] T. Ando, J. Biochem. (Tokyo) 105 (1989) 818–822.
[12] E. Prochniewicz, D.D. Thomas, Biochemistry 36 (1997) 12845–12853.
[13] T. Oda, Y. Shikata, K. Mihashi, Biophys. Chem. 61 (1996) 63–72.
[14] E.L. deBeer, A. Sontrop, M. Kellermayer, C. Galambos, G.H. Pollack, J. Cell Motility Cytoskelton 38 (1997) 341–350.

[15] K. Hatori, H. Honda, K. Matsuno, Biophys. Chem. 61 (1996) 149–152.
[16] K. Hatori, H. Honda, K. Shimada, K. Matsuno, Biophys. Chem. 75 (1998) 81–85.
[17] J.A. Spudich, S. Watt, J. Biol. Chem. 246 (1971) 4866–4871.
[18] S.V. Perry, Meth. Enzymol. 2 (1955) 582–588.
[19] Y. Okamoto, T. Sekine, J. Biochem. (Tokyo) 98 (1985) 1143–1145.
[20] S.J. Kron, Y.Y. Toyoshima, T.Q.P. Uyeda, J.A. Spudich, Meth. Enzymol. 196 (1991) 399–416.
[21] J.R. Sellers, B. Kacher, Science 249 (1990) 406–408.
[22] Y. Tanaka, A. Ishijima, S. Ishiwata, Biochim. Biophys. Acta 1159 (1992) 94–98.
[23] Y. Tajima, K. Makino, T. Hanyuu, K. Wakabayashi, Y. Amemiya, J. Muscle Res. Cell. Motility 15 (1994) 659–671.
[24] K. Wakabayashi, Y. Sugimoto, H. Tanaka, Y. Ueno, Y. Takezawa, Y. Amemiya, Biophys. J. 67 (1994) 1196–1197.
[25] K. Wakabayashi, Y. Sugimoto, H. Tanaka, Y. Ueno, Y. Takezawa, Y. Amemiya, Biophys. J. 68 (1995) 2422–2435.
[26] H.E. Huxley, A. Stewart, H. Sosa, T. Irving, Biophys. J. 67 (1994) 2411–2421.
[27] K. Matsuno, Protobiology: Physical Basis of Biology, CRC Press, Boca Raton Florida, 1989.
[28] K. Matsuno, BioSystems 46 (1998) 57–71.