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Effect of tensile force on the mechanical behavior of actin filaments

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Abstract

Actin filaments are the most abundant components of the cellular cytoskeleton, and play critical roles in various cellular functions such as migration, division and shape control. In these activities, mechanical tension causes structural changes in the double-helical structure of the actin filament, which is a key modulator of cytoskeletal reorganization. This study performed large-scale molecular dynamics (MD) and steered MD simulations to quantitatively analyze the effects of tensile force on the mechanical behavior of actin filaments. The results revealed that when a tensile force of 200 pN was applied to a filament consisting of 14 actin subunits, the twist angle of the filament decreased by approximately 20 degrees, corresponding to a rotation of approximately -2 degrees per subunit, representing a critical structural change in actin filaments. Based on these structural changes, the variance in filament length and twist angle was found to decrease, leading to increases in extensional and torsional stiffness. Torsional stiffness increased significantly under the tensile condition, and the ratio of filament stiffness under tensile force to that under no external force increased significantly on longer temporal scales. The results obtained from this study contribute to the understanding of mechanochemical interactions concerning actin dynamics, showing that increased tensile force in the filament prevents actin regulatory proteins from binding to the filament.

Keywords: Actin filament, Tensile force, Mechanical properties, Mechano-chemical interactions, Steered molecular dynamics simulation, Computational biomechanics, Cell mechanics

1 Introduction

2 The major components of the actin cytoskeleton, actin filaments, play critical roles in
3 various cellular functions, such as migration, division and shape control (Svitkina et al., 1997;
4 Watanabe and Mitchison, 2002; Pollard and Berro, 2009; Pollard and Borisy, 2003; Adachi et
5 al., 2009). In these activities, the actin cytoskeleton undergoes dynamic rearrangements
6 governed by mechanical and biochemical factors (Arber et al., 1998; Isenberg et al., 1980;
7 Pollard and Cooper, 1986; Theriot and Mitchison, 1991). In particular, changes in mechanical
8 conditions within the cells and in their surrounding environment are key regulatory factors
9 affecting the global reorganization of the actin cytoskeleton (Naruse and Sokabe, 1993;
10 Neidlinger-Wilke et al., 2001; Sato et al., 2005; Sato et al., 2000; Yamamoto et al., 2006).

11 In this reorganization process, microscopic mechanical stretching, twisting and bending
12 cause structural changes at the molecular level in single actin filaments having a
13 double-helical structure (Holmes et al., 1990; Oda et al., 2009). This structural modulation is
14 critical for inducing local cytoskeletal reorganization by interacting with a variety of
15 biochemical factors and triggering the binding of actin regulatory proteins to the filaments
16 (Hayakawa et al., 2008; McGough et al., 1997; Prochniewicz et al., 2005). Investigation of
17 the molecular mechanisms underlying how mechanical forces such as tension (Ishijima et al.,
18 1991; Shimozawa and Ishiwata, 2009) and torsional moment modulate the mechanical
19 behaviors of a single actin filament is thus important (Tsuda et al., 1996).

20 Analysis of the mechanical behaviors of actin filaments at the molecular structural level
21 is performed using numerical simulations based on the molecular dynamics (MD) method

(Chu and Voth, 2005, 2006; Pfaendtner et al., 2010). The steered MD (SMD) method (Isralewitz et al., 2001) enables control of the positions and/or velocities of some specific atoms by applying external steering forces in the appropriate direction. The SMD method is widely used to investigate the mechanical behaviors of proteins, such as stretching of the extracellular matrix (Krammer et al., 1999) and muscle proteins (Craig et al., 2002), binding/unbinding of protein-substrate complexes (Isralewitz et al., 1997; Lu et al., 1998) and adhesion proteins (Bayas et al., 2003; Vogel and Sheetz, 2006) and dissociation of phosphate from G-actin (Wriggers and Schulten, 1997, 1999). Thus, using the SMD method, we can quantitatively analyze the structural dynamics of actin molecules under external forces relevant to biological problems at the molecular level.

Our previous study (Matsushita et al., 2010) quantitatively evaluated the extensional and torsional stiffness of a single actin filament under no external forces based on an analysis of thermal fluctuations in the molecular structure using the MD method. The present study used the SMD method to investigate the effects of tensile force on mechanical behaviors of the filament. We first applied a tensile force to the molecular structure of the actin filament that was pre-equilibrated under a no-force condition. We then compared the mechanical behavior of the filament under tensile force to that under no external force.

Methods

Simulation of the filament under no external force

An actin filament structure consisting of 14 actin subunits (Fig. 1A) and equilibrated in

1 ionic solvate was obtained using the same procedure applied in our previous studies
2 (Matsushita et al., 2010). The original actin filament structure is available from the Protein
3 Data Bank (PDB code: 1MVW) (Chen et al., 2002; Holmes et al., 1990). As the actin subunits
4 in filaments under tensile force are mainly bound to ADP, ADP was placed in the individual
5 actin subunits in the filament, in which the initial coordinates of ADP were determined from
6 the ADP-bound monomer structure (PDB code: 1J6Z) (Otterbein et al., 2001). The entire
7 structure was solvated in a water box of dimensions $117 \times 118 \times 473 \text{ \AA}$, to which Na^+ and Cl^-
8 counter ions were added at a concentration of 30 mM. This system was equilibrated in an
9 NPT ensemble (pressure = 1 atm, temperature = 310 K) by performing MD simulation for 20
10 ns using NAMD 2.6 (Kale et al., 1999) with the CHARMM27 force field for proteins
11 (MacKerell et al., 1998) and the TIP3P model for water (Jorgensen et al., 1983). We applied
12 periodic boundary conditions for simulations where van der Waals interactions were
13 calculated with a cut-off distance of $13 \text{ \AA}$ and electrostatic interactions were calculated using
14 the particle mesh Ewald method (Darden et al., 1993). Free dynamics simulation under no
15 external force was then performed for 12 ns to analyze the mechanical behavior of the
16 filament.

17 *Simulation of the filament under tensile force*

18 SMD simulation under constant tensile conditions was performed by applying the
19 external force, f_{carbon} , in the $+z$ direction to all α -carbon atoms in the actin subunits G_{13} and
20 G_{14} at the plus-end and by applying the force in the $-z$ direction to those in G_1 and G_2 at the
21 minus-end (Fig. 1B). Total constant tensile force to the filament was set as $F = 200 \text{ pN}$, a

value smaller than the breaking force of the actin-actin bonds, which ranges from 320 to 600 pN (Tsuda et al., 1996).

SMD simulation under the tensile condition was performed in the same environmental setting as the simulation under the no-force condition. The simulation was performed for 12 ns to analyze the mechanical behavior of the actin filament under tensile force. Based on these MD and SMD simulations under different mechanical conditions, we investigated the effects of tensile force on filament dynamics by comparing molecular behaviors of the filaments from the perspectives of structural changes and mechanical properties.

Results

Changes in molecular structure of actin filament

Quantitative analysis of structural changes in the actin filament under different mechanical conditions was performed considering the rotational and longitudinal motions, which are critical structural motions of the filament. Rotational motion is particularly important for interaction of the actin filament with various biochemical factors (McCullough et al., 2008). In this study, filament length $L(t)$ and twist angle of the filament $\Theta(t)$ (Fig. 1C) were defined as follows (Matsushita et al., 2010):

$$L(t) = z_{\text{plus}}(t) - z_{\text{minus}}(t), \quad (1)$$

where $z_{\text{plus}}(t)$ is the position on the z -axis of the center of mass of the G-actins G_{13} and G_{14} at the plus-end, and $z_{\text{minus}}(t)$ is that of the G-actins G_1 and G_2 at the minus-end.

$$\Theta(t) = \cos^{-1} \left(\frac{\mathbf{n}_{\text{plus}}(t)}{|\mathbf{n}_{\text{plus}}(t)|} \cdot \frac{\mathbf{n}_{\text{minus}}(t)}{|\mathbf{n}_{\text{minus}}(t)|} \right), \quad (2)$$

where

$$\mathbf{n}_{\text{minus}}(t) = \mathbf{P}_{\text{G2}}(t) - \mathbf{P}_{\text{G1}}(t), \quad (3)$$

$$\mathbf{n}_{\text{plus}}(t) = \mathbf{P}_{\text{G14}}(t) - \mathbf{P}_{\text{G13}}(t), \quad (4)$$

and $\mathbf{P}_{\text{Gi}}(t)$ is the position vector of the center of mass of the G-actin G_i projected onto the x - y plane.

We monitored longitudinal thermal fluctuations in the actin filament under no external or tensile force, as shown in Figure 2A, in which changes in filament length $L(t)$ are plotted at intervals of 1 ps. For quantitative measurement of the elongation by external tensile force, the probability distribution of $L(t)$ and the approximated normal distribution curve were plotted as solid lines at intervals of 0.01 Å and broken lines, respectively (Fig. 2B). Figure 2B shows that the average ($\langle L(t) \rangle_{12\text{ ns}}$) and standard deviation ($\sqrt{\langle \Delta L^2(t) \rangle_{12\text{ ns}}}$) of length over the 12-ns period were 326.7 ± 0.6 Å and 327.3 ± 0.5 Å under no-force and tensile conditions, respectively, indicating that the elongation of approximately 0.6 Å corresponds to an extensional strain of approximately 0.2%. This was in agreement with the calculated strain of 0.2% based on extensional stiffness of 3.1 N/m (Kojima et al., 1994) and an applied external tensile force $F = 200$ pN.

Similarly, Figure 2C and Figure 2D show the rotational thermal fluctuations of the actin filament under no external and tensile forces, and the probability distribution of the twist

angle $\Theta(t)$. As shown in these figures, the average ($\langle \Theta(t) \rangle_{12\text{ ns}}$) and standard deviation ($\sqrt{\langle \Delta \Theta^2(t) \rangle_{12\text{ ns}}} = \sqrt{\langle (\Theta(t) - \langle \Theta(t) \rangle_{12\text{ ns}})^2 \rangle_{12\text{ ns}}}$) of the twist angle over the 12-ns period under no-force and tensile conditions were 179.2 ± 6.9 degrees and 159.0 ± 3.3 degrees, respectively. By applying a tensile force of 200 pN to the filament, the twist angle decreased by an average of 20.2 degrees, probably due to the structural feature of the right-handed double helix, where extensional motions of the tensile force induced coupled torsional motions. On simplifying the filament to a homogeneous rod model of a circular cross-section with a diameter equal to the magnitude of vector $\mathbf{n}_{\text{minus}}(t)$ defined in Eq. (3), the 20.2 degrees change in twist angle corresponds to a shear strain of 1.6% on the outer surface of the rod. The magnitude of the 1.6% shear strain may be significant enough to induce changes in the mechanical behavior of the filaments.

Changes in extensional and torsional stiffness

We estimated the apparent extensional stiffness ($K_{\text{ext}}^{\Delta t}(t)$) and torsional stiffness ($K_{\text{tor}}^{\Delta t}(t)$) of the actin filament from the variances of the filament length $L(t)$ and twist angle $\Theta(t)$ during the sampling-window duration Δt (Matsushita et al., 2010). The law of equipartition of energy is expressed as follows:

$$\frac{1}{2} k_{\text{ext}}^{\Delta t}(t) \langle (L(t) - \langle L(t) \rangle_{\Delta t})^2 \rangle_{\Delta t} = \frac{1}{2} k_{\text{B}} T, \quad (5)$$

$$\frac{1}{2} k_{\text{tor}}^{\Delta t}(t) \langle (\Theta(t) - \langle \Theta(t) \rangle_{\Delta t})^2 \rangle_{\Delta t} = \frac{1}{2} k_{\text{B}} T, \quad (6)$$

where $k_{\text{ext}}^{\Delta t}(t)$ and $k_{\text{tor}}^{\Delta t}(t)$ are the extensional and torsional spring constants, respectively, k_{B} is the Boltzmann constant, T is the absolute temperature, and $\langle \rangle_{\Delta t}$ indicates the average over

1 a time period $\left(t - \frac{\Delta t}{2} \leq t < t + \frac{\Delta t}{2}\right)$. If the potential energy that determines mechanical
2 behavior of the filament can be approximated as a harmonic potential in the vicinity of a
3 certain equilibrium point at a given temperature, the law of equipartition of energy is satisfied
4 irrespective of whether tensile force is applied to the actin filaments. From the spring
5 constants $k_{\text{ext}}^{\Delta t}(t)$ and $k_{\text{tor}}^{\Delta t}(t)$, the 1- μm -long apparent extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$ and the
6 apparent torsional stiffness per unit length of filament $K_{\text{tor}}^{\Delta t}(t)$ are given by

$$7 \quad K_{\text{ext}}^{\Delta t}(t) = \frac{\langle L(t) \rangle_{\Delta t}}{1 \mu\text{m}} k_{\text{ext}}^{\Delta t}(t), \quad (7)$$

$$8 \quad K_{\text{tor}}^{\Delta t}(t) = \langle L(t) \rangle_{\Delta t} k_{\text{tor}}^{\Delta t}(t). \quad (8)$$

9 Figure 3A shows the change over time in the extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$ plotted at
10 intervals of 1 ps, determined for each sampling-window duration ($\Delta t = 0.5, 1.0, 2.0, 4.0$ and
11 8.0 ns). To show the dependence of apparent stiffness on the sampling-window duration, the
12 average and standard deviation of $K_{\text{ext}}^{\Delta t}(t)$ for each sampling-window duration are plotted
13 against the sampling-window duration Δt (Fig. 3B). According to previous reports
14 (Matsushita et al., 2010), $K_{\text{ext}}^{\Delta t}(t)$ tended to decrease with increasing Δt and to converge to a
15 value. Similarly, as shown in Figure 3C and Figure 3D, torsional stiffness $K_{\text{tor}}^{\Delta t}(t)$ decreased
16 with an increase in Δt and converged to a value.

17 As shown in Figure 3B and Figure 3D, both extensional and torsional stiffness increased
18 with the application of tensile force to the actin filaments indicated by blue and red lines. To
19 quantitatively analyze an increase in stiffness due to tensile force, the ratios of filament
20 stiffness under tensile force to that under no external force, $K_{\alpha}^{\text{tensile force}} / K_{\alpha}^{\text{no external force}}$ ($\alpha = \text{ext},$

tor), are plotted in Figure 4. The ratio of torsional stiffness exhibited a large increase with increasing sampling-window duration and is expected to converge on a certain value, given a sampling-window duration long enough for the filament stiffness to converge (Fig. 3D). When compared to the longest sampling-window duration $\Delta t = 8.0$ [ns], torsional stiffness of the filament under tensile force was 3.5-fold larger than that under no external force. In contrast, the ratio of extensional stiffness exhibited no significant change. The increase in stiffness due to tensile force was thus found to differ between extensional and torsional stiffness.

Increases in torsional stiffness may be attributable to changes in the twist structure of the filament. The applied tensile force decreased the twist angle of the filament by approximately 20° , indicating that tensile force tightened the double-helical structure laterally. This constrains the rotational motions of the filament, resulting in increased torsional stiffness. In contrast, extensional stiffness showed no significant change, as filament length and longitudinal motions of the filament were constant (Fig. 2B).

Discussion

This study quantitatively analyzed the effects of tensile force on the extensional and torsional stiffness of actin filaments by performing MD and SMD simulations. When a tensile force F of 200 pN was applied to a filament consisting of 14 actin subunits, the twist angle decreased by approximately 20 degrees (Fig. 2D), representing a significant structural change for the actin filament. Given the structural changes, variances in filament length and twist

angle due to thermal fluctuations were found to decrease, leading to increases in extensional and torsional stiffness (Fig. 3B, 3D). Torsional stiffness increased significantly under the tensile force, and the ratio of filament stiffness under tensile force to that under no external force ($K_{\text{tor}}^{\text{tensile force}} / K_{\text{tor}}^{\text{no external force}}$) increased significantly with longer sampling-window duration (Fig. 4).

In order to focus on the fundamental characteristic of the dependence of the tensile force on extensional and torsional displacement, we chose the tensile condition of $F = 200$ pN as a typical example, as well as the no external force condition $F = 0$ pN. Using stiffness under the two tensile conditions of $F = 0$ pN and 200 pN, we can approximate the mechanical behavior of the filament under tensile force less than ~ 320 pN before breaking. Under the tensile force $F = 200$ pN, the extensional stiffness $K_{\text{ext}}^{200\text{pN}}$ increased 1.2-fold compared to that under tensile force $F = 0$ pN, $K_{\text{ext}}^{0\text{pN}}$ (Fig. 4). This indicates that the tensile force-extensional displacement relation exhibits nonlinear behavior. Using stiffness and displacement under the two tensile conditions, we can fit the force-displacement function as a 3rd-order polynomial. An understanding of the tensile force-torsional displacement relationship requires quantitative evaluation of the tensile force-torsional displacement coupling stiffness in the vicinity of equilibrium point $K_{\text{couple}} = F / \langle \Delta\Theta \rangle$, where F is tensile force and $\langle \Delta\Theta \rangle$ is average torsional displacement angle. This will be our next challenge, as further discussion is necessary regarding the law of equipartition of energy in consideration of the extension and torsion coupling stiffness K_{couple} .

We investigated the molecular behavior of actin filaments from mechanical and

1 structural perspectives and studied the mechanical properties involved. A number of reports
2 applying the SMD method have successfully analyzed the mechanical behaviors of various
3 biomolecules under external forces (Craig et al., 2002; Lu et al., 1998; Vogel and Sheetz,
4 2006), where changes in molecular structure are studied during transition processes
5 characterized by a thermal non-equilibrium state. In contrast, this study performed SMD
6 simulation to observe thermally equilibrated molecular behaviors under constant tensile
7 conditions, enabling quantitative analysis of thermal fluctuations of the molecules as well as
8 global structural changes, such as changes in filament length and twist angle. As these thermal
9 fluctuations in actin filaments are among the factors determining macroscopic mechanical
10 properties, analysis of thermally equilibrated molecular behaviors is necessary to achieve a
11 fundamental understanding of the mechanical properties of actin filaments.

12 Actin subunits are generally bound to ADP, ADP/Pi and ATP molecules and have three
13 nucleotide states. The dependence of these three nucleotide states on the stiffness of filaments
14 is an interesting issue from the perspective of structural biology (Pfaendtner et al., 2010). For
15 our purpose of investigating the effect of tensile force on the mechanical behavior of
16 filaments, analysis of those filaments bound to ADP is most meaningful, since filaments under
17 tensile force are mainly bound to ADP during dynamic cellular activities. For example, in the
18 process of cell migration, ATP- and ADP/Pi-actin filaments exist in the vicinity of the leading
19 edge in the lamellipodia. Conversely, ADP-actins generally exist in filament network
20 structures and stress fibers away from the leading edge. Tensile forces generated by dynamic
21 actomyosin interactions act on the filaments existing away from the leading edge. Analysis of

1 actin subunits bound to ADP is thus essential to achieving an understanding of the mechanical
2 behavior of filaments under tensile force.

3 Changes in mechanical behavior induced by external tensile force will significantly
4 affect interactions between the actin filament and various biochemical factors. Microscopic
5 tensile force along the single filament, generated by macroscopic intracellular contractile
6 forces in the actin stress fibers (Neidlinger-Wilke et al., 2001), plays an essential role in
7 interactions with a variety of actin-binding proteins, such as cofilin (Hayakawa et al., 2008).
8 In contrast, actin-binding proteins such as myosin II play a role in generating microscopic
9 mechanical tensile force, which in turn induces macroscopic contractile forces in dynamic
10 cellular processes such as cell migration (Adachi et al., 2009). The interaction between
11 mechanical and biochemical factors is thus critical for various cellular activities. However,
12 little is known about the molecular mechanisms underlying interactions between tension and
13 actin-binding proteins. Further insights into the physical mechanisms of mechanochemical
14 and chemomechanical coupling as they concern actin dynamics would thus be helpful.

15 For example, the effect of tensile force on the torsional behavior of actin filaments
16 shown in this study suggests a strong relationship to the affinity for actin regulatory proteins
17 such as cofilin. Cofilin is an actin depolymerizing and severing factor that binds along the
18 length of the filament and increases torsional (Prochniewicz et al., 2005) and bending
19 flexibilities (McCullough et al., 2008). In addition, filaments coated with cofilin show shorter
20 actin crossovers (McGough et al., 1997). Under tensile conditions, actin subunits are
21 positioned at a rotational angle of -167 degrees to the next subunit, with approximately 13

actin subunits per crossover (Meberg et al., 1998). When cofilin binds to the actin filament, the filament is locally twisted by approximately 5 degrees per subunit, from -167 degrees to -162 degrees, and the number of subunits per crossover decreases to approximately 10 (Bamburg et al., 1999).

In this study, by applying a tensile force of 200 pN to filaments with a half-turn structure consisting of 14 subunits, the twist angle was decreased by approximately 20 degrees, corresponding to a rotation of approximately -2 degrees, i.e., from -165 degrees to -167 degrees per subunit, in a direction opposite to that of the structural change when cofilin binds to the filament (Fig. 5). If the affinity of cofilin depends on the twist angle between subunits, the results obtained in this study suggest that tensile force applied to the filament prevents cofilin from binding to the filament. In addition, application of tensile force increases the torsional stiffness; that is, the variance of the twist angle decreases. The structure of proteins is not stable in one static structure, but dynamically transitions through various local metastable structures because of thermal fluctuations. Furthermore, various biochemical interactions such as protein binding occur stochastically in the thermal fluctuations. The decrease in twisting fluctuations under tensile conditions thus suggests that the binding probability of cofilin also decreases because of the decrease in twist angle. Quantitative investigation of the binding affinities of actin regulatory proteins, including cofilin, will necessitate analysis of the interactions between actin filaments and actin regulatory proteins based on free energy estimation, which will in turn enable quantification of the effects of tensile force on mechanical behaviors of the filament.

1

2 **Conflict of Interest**

3 None.

4

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Figure 1: Actin filament structure model analyzed in the SMD simulation. A) Double-helical structure of an actin filament consisting of 14 actin subunits. Each actin subunit is numbered from the minus-end as $G_1, G_2, \dots, G_{14}$. Scale bar represents 100 Å. B) An image of Region I in (A) magnified 6-fold. In SMD simulations, constant tensile forces were applied to the filament at the α -carbons in the actin subunits G_1, G_2, G_{13} and G_{14} at both ends. C) Filament length $L(t)$ was defined by Eq. (1) as the distance between the plus and minus ends of the filament. Twist angle $\Theta(t)$ was defined by Eq. (2) as the angle between the vectors n_{plus} and n_{minus} of the plus- and minus-ends, projected onto the x - y plane.

Figure 2: Structural changes during 12-ns simulations under no-force and tensile conditions.

A) Change in filament length $L(t)$. B) Average filament length is 326.7 Å under the no-force condition and 327.3 Å under the tensile condition. C) Change in twist angle $\Theta(t)$. D) Average twist angle is 179.2 degrees under the no-force condition and 159.0 degrees under the tensile condition.

Figure 3: Extensional and torsional stiffness under the no-force and tensile conditions determined for each sampling-window duration Δt . Experimentally determined extensional stiffness (Kojima et al., 1994) and torsional stiffness (Prochniewicz et al., 2005) are represented by dashed lines. A) Change in extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$. B) Average extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$ for each sampling-window duration Δt . C) Change in torsional stiffness $K_{\text{tor}}^{\Delta t}(t)$. D) Average torsional stiffness $K_{\text{tor}}^{\Delta t}(t)$ for each sampling-window duration Δt .

Figure 4: Ratios of extensional and torsional stiffness under tensile force to that under no external force [$K_{\alpha}^{\text{tensile force}} / K_{\alpha}^{\text{no external force}}$ ($\alpha = \text{ext, tor}$)], and their dependence on sampling-window duration Δt .

	Cofilin	No external force	Tensile force
Lateral view
Tensile strain	0% *	0%	0.2%
Axial view
Twist angle (degree)	-162 * +5	-165	-2 -167

* Bamburg *et al.*, 1999

Figure 5: Longitudinal and rotational motions of an actin filament induced by tensile force and binding of cofilins. When tensile force is applied to the actin filament, the filament is twisted by approximately -2 degrees per subunit from its structure under no external force. When cofilins bind to the actin filament, the filament is twisted by approximately +5 degrees per subunit from its structure under tensile force.