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Evaluation of extensional and torsional stiffness of single actin filaments by molecular dynamics analysis

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ABSTRACT

It is essential to investigate the mechanical behaviour of cytoskeletal actin filaments in order to understand their critical role as mechanical components in various cellular functional activities. These actin filaments consisting of monomeric molecules function in the thermal fluctuations. Hence, it is important to understand their mechanical behaviour on the microscopic scale by comparing the stiffness based on thermal fluctuations with the one experimentally measured on the macroscopic scale. In this study, we perform a large-scale molecular dynamics (MD) simulation for a half-turn structure of an actin filament. We analyse its longitudinal and twisting Brownian motions in equilibrium and evaluated its apparent extensional and torsional stiffness on the nanosecond scale. Upon increasing the sampling-window durations for analysis, the apparent stiffness gradually decreases and exhibits a trend to converge to a value that is close to the experimental value. This suggests that by extrapolating the data obtained in the MD analysis, we can estimate the experimentally determined stiffness on the microsecond to millisecond scales. For shorter temporal scales, the apparent stiffness is larger than experimental values, indicating that fast, local motions of the molecular structure are dominant. To quantify the local structural changes within the filament on the nanosecond scale and investigate the molecular mechanisms, such as the binding of the actin-regulatory proteins to the filaments, it is preferable to analyse the mechanical behaviour on the nanometre and nanosecond scales using MD simulation.

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1. Introduction

Several experimental studies have successfully estimated the mechanical properties of actin filaments (Kishino and Yanagida, 1988; Janmey et al., 1990), the most abundant component of cytoskeleton. They play critical mechanical roles in various cellular activities, such as migration, cell division and shape control (Svitkina et al., 1997; Watanabe and Mitchison, 2002; Pollard and Borisy, 2003; Pollard and Berro, 2009; Adachi et al., 2009). Using direct nanomanipulation techniques for single actin filaments, the 1-μm-long extensional stiffness was determined to be 0.0437 ± 0.0046 [N/m] (Kojima et al., 1994). In addition, visualization of rotational beads attached to actin filaments (Tsuda et al., 1996) and measurements of phosphorescence anisotropy (Prochniewicz et al., 2005) made it possible to evaluate the torsional stiffness per unit length of the filament, which ranges from 2.3×10^{-27} [N m^2] (Prochniewicz et al., 2005) to 8.0×10^{-26} [N m^2] (Tsuda et al., 1996).

In these experiments, the length of the measured filaments was on the micrometre scale, and the temporal scales on which the dynamic motions were observed ranged from microsecond to millisecond. However, from a more microscopic viewpoint, the filaments have a double-helix structure on the nanometre scale with a period of ~650 Å (Holmes et al., 1990; Oda et al., 2009). In the physiological environment, they are exposed to thermal fluctuations yielding fast, local motions of the structure on temporal scales ranging from picosecond to nanosecond. In other words, vibrating motions of atoms in the molecule lead to local motions of the actin subunits, which then result in longitudinal and twisting motions of the filament. These microscopic dynamics of the molecular structure are the basis of determining the macroscopic mechanical properties of the filaments when they are observed in the longer temporal scale; however, the temporal scale, which is long enough for capturing the

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mechanical properties comparable with the macroscopic properties obtained in the experiment, has not been investigated yet.

To analyse the mechanical behaviour of biomolecules at the molecular structural level, numerical simulations based on the molecular dynamics (MD) method have been extensively used and has provided us with insights into several biologically relevant problems in various biomolecular systems (Wriggers and Schulten, 1997, 1999; Lu et al., 1998; Isralewitz et al., 2001; Vogel and Sheetz, 2006). For the biological filaments that build up biological materials, MD simulations were also applied to obtain information about their biological and physical properties (Buehler and Yung, 2009). For example, the mechanical properties of amyloid fibrils associated with Alzheimer's disease have been analyzed (Paparcone et al., 2010). For the cytoskeletal filaments, the bottom–up nanomechanical analysis has been performed from the viewpoint of multi-scale mechanics. For example, this analysis was performed for the human vimentin dimmer and tetramer, which constitute the intermediate filaments (Qin et al., 2009b, a).

For the actin monomers (Dalhaimer et al., 2008; Pfaendtner et al., 2009) and filaments (Chu and Voth, 2005, 2006; Pfaendtner et al., 2010), MD simulations have successfully analyzed their structural and dynamic properties. For example, the effect of the DB loop on the nucleotide-binding cleft has been investigated (Pfaendtner et al., 2010; Chu and Voth, 2005, 2006). Using the MD method, we can quantitatively observe the structural dynamics of the actin molecules and evaluate the stiffness of actin filaments based on their thermal fluctuations.

In this study, we quantitatively evaluate the extensional and torsional stiffness of single actin filaments on the nanosecond scale by analyzing the thermal fluctuations of the molecular structure using the MD simulation, and compare the results with experimental data. We construct a model of the half-turn structure of an actin filament in an ionic solvent and analyse its longitudinal and twisting Brownian motions in equilibrium using MD simulation. We then evaluate both the extensional and torsional stiffness of the actin filament from the thermal fluctuations based on the principle of equipartition of energy.

2. Methods

2.1. Simulation procedures

The 2.0-Å resolution X-ray crystallographic structure of actin filament was obtained from the Protein Data Bank (PDB code: 1MVW) (Holmes et al., 1990; Chen et al., 2002). From an original structure derived from rabbit skeletal muscle with six myosins, an actin filament structure consisting of 14 actin subunits was extracted as shown in Fig. 1. Then, ADP was placed into the individual actin subunits in the filament, in which the coordinates of the ADP were obtained from the ADP-bound monomer structure (PDB code: 1J6Z) (Otterbein et al., 2001). The actin filament structure of dimensions ($97\times98\times423$ Å^3) was placed into a water box of dimensions ($117\times118\times473$ Å^3), in which we utilized the TIP3P model for water molecules (Jorgensen et al., 1983). To the water box, Na^+ and Cl^- counter ions were added to reach a concentration of 30 mM. The total system consisted of 496,434 atoms; 80,836 actin filament atoms, 415,212 water atoms, 236 Na^+ ions and 150 Cl^- ions.

MD simulation was performed using NAMD 2.6 (Kale et al., 1999) with the CHARMM27 force field for proteins (MacKerell et al., 1998). We used periodic boundary conditions for simulations in which the van der Waals interactions were calculated with a cutoff of 13 Å and the electrostatic interactions were calculated using the particle mesh Ewald method (Darden et al., 1993). A multiple time-stepping algorithm (Schlick et al., 1999) was used with a 2-fs step.

MD simulations were performed in an NPT ensemble (pressure = 1 atm, temperature = 310 K), in which the pressure was controlled by the hybrid Nosé–Hoover Langevin piston method (Hoover, 1985; Martyna et al., 1992) and the temperature was controlled using Langevin dynamics. After relaxing the water molecules for 200 ps keeping all the protein atoms fixed, a free dynamics simulation for the entire system was performed for 12 ns to obtain the equilibrium structure.

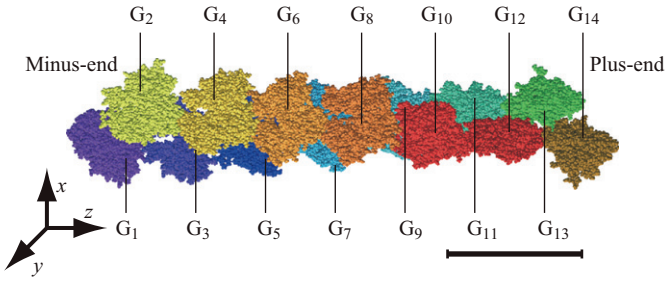

Fig. 1. Double-helix structure of an actin filament consisting of 14 actin subunits. The total protein size is 80,836 atoms. The Cartesian coordinate axis z is along the filament axis, and the orthogonal axes are x and y. Each actin subunit is numbered from the minus-end as G_1, G_2,…,G_{14}. The size of the scale bar is 100 Å.

2.2. Evaluation of stiffness

Length of the actin filament structure, $L(t)$, is defined as

$$L(t)=z_{\mathrm{plus}}(t)-z_{\mathrm{minus}}(t) \tag{1}$$

where $z_{\mathrm{plus}}(t)$ is the position in the z-axis of the centre of mass of G-actins, G_{13} and G_{14}, at the plus-end, and $z_{\mathrm{minus}}(t)$ is that of the G-actins, G_1 and G_2, at the minus-end, as shown in Fig. 2 (left).

Twist angle of the actin filament structure, $\Theta(t)$, is defined as

$$\Theta(t)=\cos^{-1}\left(\frac{\boldsymbol{n}_{\mathrm{plus}}(t)}{|\boldsymbol{n}_{\mathrm{plus}}(t)|}\bullet\frac{\boldsymbol{n}_{\mathrm{minus}}(t)}{|\boldsymbol{n}_{\mathrm{minus}}(t)|}\right) \tag{2}$$

where

$$\boldsymbol{n}_{\mathrm{minus}}(t)=\boldsymbol{P}_{\mathrm{G2}}(t)-\boldsymbol{P}_{\mathrm{G1}}(t) \tag{3}$$

$$\boldsymbol{n}_{\mathrm{plus}}(t)=\boldsymbol{P}_{\mathrm{G14}}(t)-\boldsymbol{P}_{\mathrm{G13}}(t) \tag{4}$$

and $\boldsymbol{P}_{\mathrm{G}i}(t)$ is the position vector of the centre of mass of G-actin G_i projected onto the x-y plane, as shown in Fig. 2 (right). For the analysis, the filament length $L(t)$ and twist angle $\Theta(t)$ are determined by aligning the longitudinal axis of the filament along the z-direction in each frame.

Using the law of equipartition of energy, the extensional spring constant $k_{\mathrm{ext}}^{\Delta t}(t)$ and torsional spring constant $k_{\mathrm{tor}}^{\Delta t}(t)$ of the filament can be estimated from the variances of the filament length $L(t)$ and twist angle $\Theta(t)$ during the sampling-window duration Δt, as

$$\tfrac{1}{2}k_{\mathrm{ext}}^{\Delta t}(t)\langle(L(t)-\langle L(t)\rangle_{\Delta t})^2\rangle_{\Delta t}=\tfrac{1}{2}k_{\mathrm{B}}T \tag{5}$$

$$\tfrac{1}{2}k_{\mathrm{tor}}^{\Delta t}(t)\langle(\Theta(t)-\langle\Theta(t)\rangle_{\Delta t})^2\rangle_{\Delta t}=\tfrac{1}{2}k_{\mathrm{B}}T \tag{6}$$

where k_{B} is the Boltzmann constant, T is the absolute temperature and $\langle\ \rangle_{\Delta t}$ indicates the average over a time $(t-(\Delta t/2)\le t<t+(\Delta t/2))$. In this study, the spring constants $k_{\mathrm{ext}}^{\Delta t}(t)$ and $k_{\mathrm{tor}}^{\Delta t}(t)$ of the filament for $\Delta t=0.5$, 1.0, 2.0, 4.0, 8.0 and 16.0 ns were evaluated based on the MD simulations.

In this study, we evaluate the stiffness by using the conventional units, as used in previous experimental studies (Kojima et al., 1994; Prochniewicz et al., 2005). Extensional stiffness has been calculated as the value per 1-μm length, and torsional stiffness as the value per unit length. From the spring constants $k_{\mathrm{ext}}^{\Delta t}(t)$ and $k_{\mathrm{tor}}^{\Delta t}(t)$, the 1-μm-long apparent extensional stiffness, $K_{\mathrm{ext}}^{\Delta t}(t)$, is given by

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

and the apparent torsional stiffness, $K_{\mathrm{tor}}^{\Delta t}(t)$, per unit length of filament is given by

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

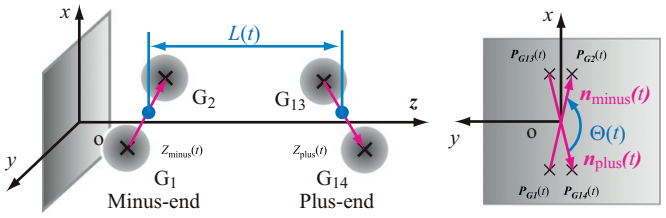

Fig. 2. Definition of filament length and twist angle. The filament length $L(t)$ is defined by Eq. (1) as the distance between the plus- and minus-ends of the filament (left). The twist angle $\Theta(t)$ is defined by Eq. (2) as the angle between the vectors of the plus- and minus-ends (right).

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