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Biophysical characterization of cofilin-induced extension–torsion coupling in actin filaments

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ABSTRACT

Cofilin makes the actin filament flexible and thermally unstable by disassembling the filament and inducing bending and torsional compliance. Actin monomers bound to cofilin are able to chemically and mechanically interact in response to external forces. In this study, we performed two molecular dynamics tensile tests for actin and cofilactin filaments under identical conditions. Surprisingly, cofilactin filaments were found to be twisted, generating shear stress caused by torsion. Additionally, analysis by plane stress assumption indicated that the extension–torsion coupling effect increases the amount of principal stress by 10%. Using elasticity and solid mechanics theories, our study elucidates the role of cofilin in the disassembly of actin filaments under tensile forces.

1. Introduction

Actin filaments are abundant components of the cellular cytoskeleton, which play various roles in shape control, cell migration, and cell division (Matsushita et al., 2010; Pollard and Berro, 2009; Pollard and Borisy, 2003; Svitkina et al., 1997; Watanabe and Mitchison, 2002). A thorough understanding of the characteristics of actin filaments is required for elucidation of their cellular functions. Additionally, actin filaments are the most common proteins in the cytoplasm (Pollard, 1990). Therefore, numerous studies of their mechanical properties and dynamics have been undertaken.

Many types of actin-binding proteins are involved in the regulation of actin filament structure and activity (Nishida et al., 1984; Sheterline et al., 1998). Cofilin, one of the essential actin regulatory proteins, promotes actin filament fragmentation by freeing barbed ends for polymerization, thereby increasing the motility of the actin filament (Carlsson, 2006; Enrique, 2009; Pavlov et al., 2007). During disassembly, cofilin binds to actin filaments to increase their torsional flexibility by inducing twisting behavior, thereby affecting their mechanical properties and dynamics (McCullough et al., 2011; McGough et al., 1997; Prochniewicz et al., 2005).

Actin and cofilactin filaments are exposed to tensile forces, as these filaments serve as tracks for myosin molecular motors during muscle contraction and cell migration. (Matsushita et al., 2011, 2012; Vilfan, 2009). It is known that cofilin plays a major role in regulating torsional dynamics of actin filaments; however, the extension–torsion coupling effect of cofilin on actin filaments has not been investigated to date. Previous reports by Matsushita et al. (2010, 2011 and 2012) focused on effects of tensile force on actin filaments and extension–torsion coupling of actin filaments, but did not analyze the effects of cofilin binding on actin filaments. The force applied by myosin is very small as 0.025 nN on actin filament (Craig Erin et al., 2012). The biological force acted on actin filament is quite small. As in these studies, SMD study using actin filaments used pulling force with $F=200$ pN (Matsushita et al., 2011).

An understanding of coupling effects is highly valuable in several fields. For instance, in the field of aerospace engineering, it is known that the bending–torsion coupling interaction has a noticeable impact on cascade flutter boundary (Bendiksen and Friedmann, 1980), and that the coupling effect may be controlled by the degree of anisotropy of the individual layers and the number of piles in the composite (Whitney, 1969). Furthermore, this effect and the associated equations of pre-twisted beams have been widely analyzed (Jiang and Henshall, 2001; Krenk, 1983). The conformation of actin filament is very similar to pre-twisted beams which is analyzed in the engineering field (Galkin et al., 2011; Holmes, 1990). Some researches have reported that possibilities of coupling effect with bending or tensile extension on actin filament (De La Cruz et al., 2010; Matsushita et al., 2011). Also binding of cofilin on actin filament affected winding of actin filament without tensile force, which may imply different behavior of

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cofilin bound actin with tensile force compared to pure actin filament (Matsushita et al., 2011).

In molecular dynamics (MD) simulations, all-atom simulations have computational limitation due to their computational inefficiency in large systems (Perez et al., 2012; Romo and Grossfield, 2011; Rueda et al., 2007). To resolve this issue, coarse-grained methods have been highlighted as an alternative method, as these enable efficient protein analysis, requiring less computational power and producing more reliable results (Ayton and Voth, 2007; Chu and Voth, 2006; Monticelli et al., 2008, Pfaendtner and Voth, 2008; Tirion, 1996). In particular, the coarse-grained force field, MARTINI, which has been actively applied recently, has provided suitable models (Lu et al., 2014; Marrink and Tieleman, 2013). MARTINI uses a single pseudo-atom for expressing an average of four heavy atoms with polar (*P*), nonpolar (*N*), apolar (*C*), and charged (*Q*) at interaction sites (Marrink et al., 2007). Our previous study with actin filament also applied MARTINI force field and elastic network model (ENM) to find effect of cofilin binding on actin filament (Kim et al., 2015).

There have been many studies investigating protein dynamics and mechanical behaviors using elasticity and solid mechanics theories. Euler–Bernoulli's beam theory has been applied to determine the characteristics of beam shaped fiber structures such as amyloid fibrils, and microtubules (Choi et al., 2015; Deriu et al., 2010; Xu et al., 2010; Yoon et al., 2011, 2014). In addition, in both experimental and MD simulation-based analyses of their mechanical properties, such as Young's moduli and bending rigidities, actin and cofilactin filaments are considered to be elastic and continuum beams or rods (Janmey et al., 2001; Kim et al., 2015; McCullough et al., 2008; Prochniewicz et al., 2005). These analyses have produced highly reliable results that have been consistent with data from other experiments. Various studies of proteins applying solid mechanics are widely accepted as useful and important for the elucidation of the biophysical properties and origin of their constitution of these molecules.

In the present study, we performed steered MD (SMD) simulations to excite tensile forces on actin and cofilactin filaments, and analyzed twisting behaviors relating to this extensional force. During the experiments, significant differences in twist angle changes due to coupling effects between actin and cofilactin filaments were observed. We attributed these dissimilarities to the interaction between actin monomers and decorated cofilins and verified that cofilin plays a significant role in disassembling actin filament by means of coupling effect. As in previous reports, assumptions of elastic beam were applied in this study. Additionally, we used elasticity and solid mechanics theories to demonstrate that cofilins exert an extension–torsion coupling effect, increasing the maximum stress by 10% and changing the inclined angle to be normal to the tangential line of the actin monomer junction. Additionally, we suggest that there is a relationship between force and twist angle in the elastic zone of the cofilactin filament.

2. Methods

In order to characterize the dynamics of actin and cofilactin filaments under the same tensile test conditions, a protein data bank (PDB) code acquired in the same environment was selected. We used PDB code of 3J0S for preparing two materials, one for actin and the other for cofilactin filament. The protein data was used to generate a 9.0 Å resolution cryo-electron microscopic three-dimensional reconstruction of cofilin-decorated actin filaments containing 12 actin monomers, with 12 cofilins bound to each monomer (Galkin et al., 2011). The 3J0S protein data was directly used for preparing the cofilactin filament and pure actin filament materials. Only actin subunits were used, after removing all bound cofilins, for preparing the actin filament material.

The purpose of coarse-grained molecular dynamics is to perform simulations with higher computing efficiency and result accuracy in diverse biomolecular systems (Marrink and Tieleman, 2013). In particular, MARTINI provides suitable coarse-grained modeling methods based on four-to-one mapping (Gautieri et al., 2010; Marrink et al., 2007). We mapped the proteins applying MARTINI 2.1P version force field, which considers the polarized effect of protein and water molecules, with GROMACS 4.6.5 package (Hess et al., 2008; Yesylevskyy et al., 2010a). Setting periodic boundary condition (PBC) with triclinic unit cell energy minimization (EM) molecular dynamics simulation was performed in vacuum state for 5000 steps. Saline solution was subsequently filled with MARTINI polarizable water molecules and randomly placed Na^+ ions and Cl^- ions to obtain a neutralized system with 0.1 M salt concentration (Yesylevskyy et al., 2010b). Then, the system was treated with EM process using steepest descent algorithm until the number of calculations reached 5000 times or the maximum force acting on the system was less than 100 kJ/mol/nm. Temperature and pressure were stabilized at 310 K and 1.0 bar respectively under NVT and NPT ensemble conditions, using velocity rescaling temperature coupling method with a stochastic term and Berendsen pressure coupling method (Berendsen et al., 1984; Bussi et al., 2007). Finally, equilibrium (EQ) dynamic simulations of actin and cofilactin filaments were carried out using leap-frog algorithm in NVT ensemble conditions for 40 ns, with a time step at 20 fs, saving trajectory and energy every 2 ps (Hockney et al., 1974).

Tensile tests for the two filaments were simulated using SMD after obtaining saturated values of root mean square deviation, which indicated that the proteins were well equilibrated. We reconstructed the system boxes for extension, filled saline solution, and processed EM. After stabilizing temperature and pressure, the proteins were extended constraining heavy atoms of two actin monomers A and B (Fig. 1(a)) and pulling center of mass (COM) of K and L in a constant velocity. Pulling velocity was fixed to 0.5 nm/ns, and calculations were performed until the filaments were completely fractured. Tensile tests were performed three times each for two different filaments.

3. Results

3.1. Changes in twist angles

The main focus of this work was to investigate torsional behaviors when the protein is subjected to tensile force only at the end of the filament. We grouped six pairs of monomers, AB, CD, EF, GH, IJ, and KL, and calculated twist angles (θ) from vectors of each contiguous pair in Fig. 1(b). Each vector was obtained from COM of each monomer in one pair, e.g. from COM of A to COM of B and so on.

As previously mentioned, actin and cofilactin filaments were considered as an elastic beam or rod because of their mechanical properties such as persistence length (~10 μm) and

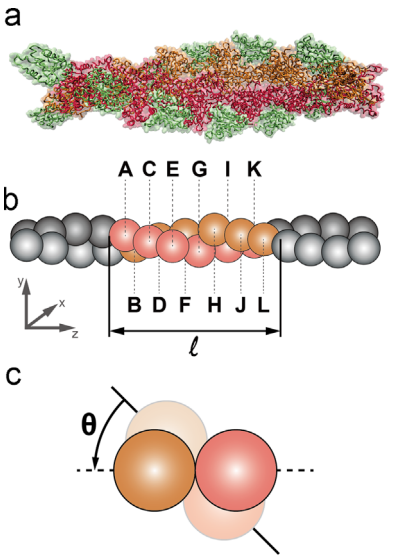

Fig. 1. (a) Structural view of cofilactin used in this study. (PDB: 3J0S). Actin monomers are colored in red and orange, cofilin is colored in green. (b) Schematic of actin filament. Chain A to L represents the region considered in the present study (pink and orange). (c) Measuring twist angle (θ) between two pairs of monomers. Twist angle is measured between vectors connecting the centers of mass of two monomers of each pair. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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