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Molecular Origins of Cofilin-Linked Changes in Actin Filament Mechanics

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Abstract

The actin regulatory protein cofilin plays a central role in actin assembly dynamics by severing filaments and increasing the concentration of ends from which subunits add and dissociate. Cofilin binding modifies the average structure and mechanical properties of actin filaments, thereby promoting fragmentation of partially decorated filaments at boundaries of bare and cofilin-decorated segments. Despite extensive evidence for cofilin-dependent changes in filament structure and mechanics, it is unclear how the two processes are linked at the molecular level. Here, we use molecular dynamics simulations and coarse-grained analyses to evaluate the molecular origins of the changes in filament compliance due to cofilin binding. Filament subunits with bound cofilin are less flat and maintain a significantly more open nucleotide cleft than bare filament subunits. Decorated filament segments are less twisted, thinner (considering only actin), and less connected than their bare counterparts, which lowers the filament bending persistence length and torsional stiffness. Using coarse-graining as an analysis method reveals that cofilin binding increases the average distance between the adjacent long-axis filament subunit, thereby weakening their interaction. In contrast, a fraction of lateral filament subunit contacts are closer and presumably stronger with cofilin binding. A cofilactin interface contact identified by cryo-electron microscopy is unstable during simulations carried out at 310 K, suggesting that this particular interaction may be short lived at ambient temperatures. These results reveal the molecular origins of cofilin-dependent changes in actin filament mechanics that may promote filament severing.

Introduction

Actin filament assembly plays essential functional roles in cell motility, cell division, endocytosis, and intracellular transport. Assembled filaments form networks that help determine the shape and mechanical integrity of cells. Numerous actin-binding proteins facilitate actin filament assembly, disassembly, and organization.[1–5] Among these proteins, cofilin plays a crucial role in actin (dis) assembly dynamics through severing filaments, which accelerates filament turnover by increasing the number of filament ends, where subunits both bind to and dissociate from filaments.

The mechanism by which cofilin severs actin filaments has been explored extensively using biochemical, structural, and computational approaches.[6–31] Changes in actin filament bending and twisting mechanics[16,24,27,28] appear to be related to filament severing by vertebrate cofilin.[28] Existing data favor a model in which stress accumulation promotes severing at regions with mechanical discontinuities, such as near boundaries between stiff, bare actin and compliant, cofilin-decorated segments.[26,28,30] This

model explains why partially decorated filaments fragment more readily than bare filaments.[17,18,23]

Cofilin alters the (average) structure of actin filaments.[6,9,10,20,22,27,29,31] Reconstruction of negatively stained samples reveals that cofilin occupancy changes the average filament twist by ~5° per subunit[6] and may induce a (~12°) change in the average subunit tilt.[9] As a result, longitudinal filament contacts are reorganized,[10–12,21,24,25] which increases the fluctuation dynamics of the C-terminus and the DNase I binding loop located within subdomain 2.[15,16] Longitudinal contacts between subdomains 3 and 4 of neighboring subunits along the long-pitch helix, however, are minimally affected.[29] Lateral filament contacts are also reorganized.[8,14] Electron microscopy (EM) image analysis of negatively stained samples[12] and time-resolved spectroscopic studies[16] suggest that cofilin-dependent structural changes reflect redistribution of equilibrium conformers populated by bare actin, though evidence for novel conformations exists.[16]

Cofilin binding and subsequent conformational changes depend strongly on the chemical state of the actin-bound nucleotide and, therefore, cofilactin conformational changes are linked to occupancy of the high-affinity nucleotide-binding site.[10,20,22,29] Solution studies suggest that the nucleotide clefts of cofilactin filament subunits adopt a more open conformation than those of bare actin filament subunits, resembling that of free actin monomers.[10,20] Three-dimensional reconstructions of filaments imaged by cryo-EM provide structural information that reveals that the cofilactin nucleotide cleft conformation is in a more open conformation than that of either G- or bare F-actin.[29]

Computational studies have complemented actin and cofilactin experiments by providing quantitative connections between the properties, structure, and interactions of actin filaments with atomistic detail.[27,31–35] Chu and Voth[32] investigated how the persistence length of undecorated actin filaments varies with changes in D-loop conformation using molecular dynamics (MD) simulations. Pfaendtner *et al.*[33] refined this study using improved filament structures[36] and found that unfolding of the D-loop increased the persistence length regardless of the state of the bound nucleotide. A subsequent paper by Pfaendtner *et al.*,[27] in which MD simulations were performed starting from an early structure of cofilactin,[12] showed that the persistence length of the cofilin-decorated filament is lower than that of the bare filament due to weakened longitudinal interactions. This study predicts that cofilin binding would cause the rearrangement of subdomain 2 of actin, resulting in weakened longitudinal interactions in the actin filament. This prediction was confirmed by the most recent high-resolution cofilactin filament structure where subdomain 2 rotates inward towards the other strand.[29] Wong and Sept deduced structural information about cofilin-decorated actin filaments using molecular docking and MD.[31] More recently, the extensional and torsional stiffness of undecorated actin filaments have been evaluated using MD simulations.[34,35] These studies demonstrate how local conformational changes can propagate to affect macroscopic filament properties.

In this study, we extend the exploration of cofilin-induced changes in the persistence length of actin filaments begun by Pfaendtner *et al.*[27] using a more recently released cofilactin structure.[29] We employ MD simulations and coarse-grained (CG) analysis to evaluate how cofilin-induced changes in actin structure affect both the bending and twisting dynamics of the filament and to identify the molecular determinants of this behavior and how these changes are modulated by cofilin binding. This work also advances previous investigations of actin filament mechanics and cofilin severing based on continuum mechanics interpretations.[24,28]

Results and Discussion

Cofilin binding opens the nucleotide-binding cleft of filament subunits by changing the dihedral angle twist

Cofilin binding alters the actin filament structure at both the subunit and filament level. When comparing the structures of subunits in the bare and cofilin-decorated filaments (hereafter called the *cofilactin filament*), we consider the local-scale, relatively rigid part of each actin subdomain:[37] residues 5–33, 80–147, and 334–349 of SD1; residues 34–39 and 52–59 of SD2; residues 148–179 and 273–333 of SD3; and residues 180–219 and 252–262 of SD4. The centers of geometry (COGs) for these residues are labeled as R1, R2, R3, and R4 (shown in Fig. 1a). Most of the equilibrated structural parameters of filament subunits (intersubunit bond distances, angle, and dihedral; Table 1) do not deviate significantly from the crystal structures.[29,36] However, two differences emerge between the cofilactin and bare filament subunits. The equilibrated R2–R1–R3–R4 dihedral angles differ significantly (10.27° ± 3.41° and 25.73° ± 3.71° for bare actin and cofilactin, respectively. This dihedral angle R2–R1–R3–R4 is one measure of how widely the nucleotide-binding cleft opens, as shown in Fig. 1b). In the cryo-EM reconstruction[29] of cofilactin, subdomains 1 and 2 of the actin subunits rotate toward the adjacent strand to avoid steric clashes and the angle between vector R1–R2 and the twist axis of the filament differs by 16° between the two filaments as a consequence of this reorientation (Table 1).