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Competitive displacement of cofilin can promote actin filament severing

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ABSTRACT

Cofilin is an essential actin filament severing protein that functions in the dynamic remodeling of the actin cytoskeleton. Filament severing activity is most efficient at sub-stoichiometric cofilin binding densities (i.e. <1 cofilin per actin filament subunit), and peaks when the number density of boundaries (i.e. junctions) between bare and cofilin-decorated segments is maximal. A model in which local topological and mechanical discontinuities lead to preferential fragmentation at boundaries accounts for available experimental data, including direct visualization of cofilin and actin during real-time severing events. The boundary-severing model predicts that ligands (e.g. other actin-binding proteins) that compete with cofilin for actin filament binding and modulate cofilin occupancy on filaments will alter the bare-decorated segment boundary density, and thus, the filament severing activity of cofilin. Here, we directly test this model prediction by evaluating the effects of phalloidin and myosin, two ligands that compete with cofilin for filament binding, on the actin filament binding and severing activities of cofilin. Our experiments demonstrate that competitive displacement of cofilin lowers cofilin occupancy and promotes severing when initial cofilin occupancy is high (i.e. >50%). Even in the presence of competitive ligands, maximum severing activity occurs when cofilin-decorated boundary density is highest, consistent with preferential fragmentation at boundaries. We propose a general "severodyne" framework for the modulation of cofilin-mediated actin filament severing by small molecule or actin-binding protein ligands that compete with cofilin for actin filament binding.

1. Introduction

Remodeling of the actin cytoskeletal network is vital for many fundamental cellular processes including cell division, growth, and motility [1–3]. Cofilin is an essential actin regulatory protein that binds and severs actin filaments [4–7]. Human cofilin binds vertebrate actin filaments cooperatively [8–10], and a lattice model with nearest neighbor cooperative binding interactions reliably describes the equilibrium [11] and kinetics [12,13] of binding.

Filaments partially decorated with cofilin sever more readily than bare or saturated filaments [5,7,11,14–20]. Severing activity is maximal at half-stoichiometric cofilin occupancy (i.e. binding density, $v_{cof} = 0.5$ cofilin per actin filament subunit) [5,18,20], where the number of boundaries between bare and cofilin-decorated filament regions is highest [5,7,18]. Direct real-time visualization of cofilin-mediated severing events indicates that severing occurs preferentially at or near boundaries [18,20].

Central to understanding cofilin-mediated filament severing *in vivo* is knowledge of how other actin regulatory proteins modulate cofilin binding and severing activity. Actin regulatory proteins that compete with cofilin for filament binding can modulate cofilin binding density along filaments and, therefore, severing activity. The boundary-severing mechanism specifically predicts that displacement of bound cofilin will either increase or decrease filament severing, depending on the initial cofilin occupancy. Because severing activity peaks at a cofilin binding density of ~0.5, competitive displacement of bound cofilin is predicted to increase the boundary density and promote severing when initial cofilin occupancy is high ($v_{cof} > 0.5$) but lower the boundary density and reduce severing when initial occupancy is low ($v_{cof} < 0.5$).

In this study, we directly test this model prediction through evaluation of the effects of phalloidin and myosin on cofilin binding and severing of actin filaments. At high cofilin occupancy ($v_{cof} = 0.9$), inclusion of either phalloidin or myosin displaces cofilin, which increases the boundary density and promotes filament severing. Filaments partially decorated with cofilin and competing ligands sever maximally when the cofilin binding density is approximately half-saturating ($v_{cof} \sim 0.5$), consistent with preferential severing at boundaries of cofilin-decorated segments.

Abbreviations: L_{avg}, average filament length; v_{cof}, cofilin binding density; NEM, N-ethylmaleimide; S1, subfragment 1; XAip1, *Xenopus* Actin-interacting protein 1.

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2. Materials and methods

2.1. Protein expression, purification, and labeling

All reagents were the highest purity commercially available and purchased from Sigma–Aldrich, unless otherwise noted. Vertebrate skeletal muscle actin was purified from rabbit back and leg muscle, labeled (efficiency > 0.85) with pyrenyl-iodoacetamide, Alexa 488-, or Alexa 594-succimidyl ester (Molecular Probes), and filtered at 4 °C over Sephacryl S-300 (equilibrated in 0.2 mM ATP, 0.1 mM $CaCl_2$, 0.5 mM DTT, 1 mM NaN_3, 2 mM Tris–HCl, pH 8.0) as described [17,18,21]. Ca^{2+}-actin was converted to Mg^{2+}-actin on ice with 0.2 mM EGTA and 20–80 μM $MgCl_2$ before polymerizing with 0.1 vols of 10× polymerizing buffer (500 mM KCl, 20 mM $MgCl_2$, 200 mM imidazole, pH 6.8) and freshly dissolved DTT, yielding a solution of Mg^{2+}-actin filaments in $KMI_{6.8}$ buffer (50 mM KCl, 2 mM $MgCl_2$, 2 mM DTT, ~0.2 mM ATP, ~1 mM NaN_3, 20 mM imidazole, pH 6.8).

Recombinant human nonmuscle cofilin-1 was expressed and purified as described [11,17]. Cofilin concentration was determined spectroscopically by the Edelhoch method [22]. Purified rabbit skeletal muscle myosin S1 was provided as a generous gift from David D. Thomas (University of Minnesota), and labeled with N-ethylmaleimide (Thermo Fisher Scientific) as described [23]. Phalloidin stock (1 mM) was prepared in methanol.

2.2. Equilibrium binding assays

Cofilin binding to pyrene-labeled actin filaments was assayed by fluorescence and cosedimentation [11,12] (Mc2008, 2011). Equilibrium binding of human cofilin to 2.5 μM pyrene-labeled actin filaments (in the presence and absence of the indicated phalloidin or NEM myosin S1 concentrations) was monitored by fluorescence (λ_{ex} 366 nm, λ_{em} 407 nm) with a SpectraMax Gemini XPS plate reading fluorimeter (Molecular Devices). Measurements were made at 22 °C in $KMI_{6.8}$ buffer. Observed intensities were converted to cofilin and competitor binding densities as described [11,17]. Cofilin binding to Alexa-labeled actin filaments used in microscopy assays was validated by cosedimentation [11,17]. No detectable depolymerization occurred in the presence of cofilin under our conditions, as reported [16].

2.3. Fluorescence microscopy

Alexa 488 or Alexa 594-labeled actin filaments equilibrated with cofilin and/or competitors (myosin or phalloidin) were diluted in $KMI_{6.8}$ buffer supplemented with 20 μg/mL catalase, 100 μg/mL glucose oxidase, and 10 mM glucose and free cofilin (and competitor, where appropriate, at corresponding concentrations to ensure initial occupancy did not change [17,18]) and immobilized on poly-L-lysine treated slides. Filaments were imaged at room temperature (~22 °C) using a Till iMic digital microscope system equipped with a 100× objective (Olympus), cooled Andor iXon897 EMCCD camera, and LiveAcquisition software. Digital images were processed using ImageJ software (NIH). Single actin filaments longer than 6 pixels (0.07 μm/pixel) were detected and reconstructed using a custom Matlab script, with manual user verification to exclude bundles [17,18,21]. The average contour lengths and bending persistence lengths of actin filaments at varying cofilin, phalloidin, and myosin occupancies were determined from analysis of 100 to 500 individual filaments (~20 images) in each data set, as described in detail [17,18,21].

3. Results and discussion

3.1. Phalloidin and myosin compete with cofilin for actin filament binding

Phalloidin and myosin compete with cofilin for actin filament binding [9,24–27]. Myosin and cofilin binding sites are overlapping (comparison of Refs. [28,29]). Phalloidin and cofilin binding sites are distinct, but linked allosterically [9,27,30,31]. These two ligands therefore provide distinct classes of chemical linkage for competing cofilin from actin filaments.

We performed competitive equilibrium binding assays to determine conditions under which myosin or phalloidin displace cofilin from actin to yield filaments with a range of cofilin and myosin or phalloidin binding densities. Competitive displacement of cofilin from actin filaments at high initial cofilin occupancy (v_{cof} > 0.9) was measured by pyrene fluorescence enhancement as either phalloidin or myosin was titrated over a broad concentration range (Fig. 1A and B). Because phalloidin and NEM-treated myosin bind actin filaments more tightly than cofilin [11,12,26,32,33], they effectively displace bound cofilin and allow us to identify experimental conditions for evaluation of severing activity in which the cofilin binding density can be precisely controlled through competition.

3.2. Phalloidin and myosin promote filament severing by displacement of cofilin at high initial occupancy

Severing lowers the average actin filament length (L_{avg}) at steady state and provides a quantitative metric of filament severing activity [18,34,35] As previously reported [18,20], filaments half-saturated with cofilin (v_{cof} = 0.5) possess the maximum allowable boundary density [5,13], sever with the greatest frequency [18,20], and thus display the shortest observed L_{avg} (Fig. 2A and C–E). Filaments with low (i.e. $v_{cof} \rightarrow 0$) or high (i.e. $v_{cof} \rightarrow 1.0$) cofilin binding densities contain a low bare-decorated boundary

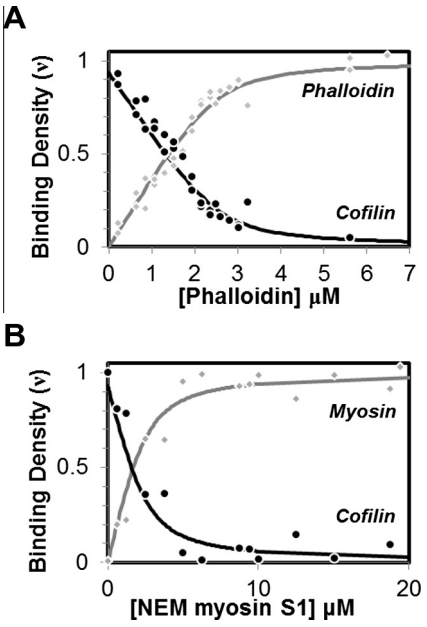

Fig. 1. Phalloidin and myosin dissociate cofilin from actin filaments. (A) Cofilin (black) and phalloidin (gray) binding densities derived from equilibrium fluorescence titrations. (B) Cofilin (black) and NEM-myosin S1 (gray) binding densities derived from fluorescence data. Solid lines through the data represent the best fits of the data to a two ligand, linked equilibrium competitive binding model [45], where phalloidin and myosin have no binding cooperativity.