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Original Contribution

Taurine chloramine-induced inactivation of cofilin protein through methionine oxidation

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ABSTRACT

Cofilin regulates reorganization of actin filaments (F-actin) in eukaryotes. A recent finding has demonstrated that oxidation of cofilin by taurine chloramine (TnCl), a physiological oxidant derived from neutrophils, causes cofilin to translocate to the mitochondria inducing apoptosis (F. Klamt et al. *Nat. Cell Biol.* **11**:1241–1246; 2009). Here we investigated the effect of TnCl on biological activities of cofilin *in vitro*. Our data show that TnCl-induced oxidation of recombinant human cofilin-1 inhibits its F-actin-binding and depolymerization activities. Native cofilin contains four free Cys and three Met residues. Incubation of oxidized cofilin with DTT does not lead to its reactivation. A double Cys to Ala mutation on the two C-terminal Cys shows similar biological activities as the wild type, but does not prevent the TnCl-induced inactivation. In contrast, incubation of oxidized cofilin with methionine sulfoxide reductases results in its reactivation. Phosphorylation is known to inhibit cofilin activities. We found that Met oxidation also prevents phosphorylation of cofilin, which is reversed by incubating oxidized cofilin with methionine sulfoxide reductases. Interestingly, intact protein mass spectrometry of the oxidized mutant indicated one major oxidation product with an additional mass of 16 Da, consistent with oxidation of one specific Met residue. This residue was identified as Met-115 by peptide mapping and tandem mass spectrometry. It is adjacent to Lys-114, a known residue on globular-actin-binding site, implying that oxidation of Met-115 disrupts the globular-actin-binding site of cofilin, which causes TnCl-induced inactivation. The findings identify Met-115 as a redox switch on cofilin that regulates its biological activity.

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Introduction

Actin is an ATP/ADP-binding protein and one of the most abundant proteins in eukaryotic cells. Under physiological salt conditions, the ATP-loaded monomeric actin (globular actin or G-actin) can spontaneously polymerize into double-helical filaments (F-actin) that serve as the core of the microfilament system. ATP-G-actin associates to one end (barbed end or plus end) of the filaments resulting in rapid growth of the filaments. ATP on the incorporated monomer undergoes hydrolysis to release phosphate (Pi), with ADP/Pi-bound actin as the intermediate. ADP-G-actin dissociates at the other end (pointed end or minus end) of the filaments, which causes shrinking of the filaments (Fig. 1). This polymerization and depolymerization of F-actin leads to dynamic turnover of the filaments, which is essential for most cellular processes that involve movement, including cell motility, cell division, cytokinesis, and endocytosis [1,2].

The dynamics of actin in cells is tightly regulated by the actin-depolymerizing factor (ADF)/cofilin family of proteins [1,2]. Cofilin has much higher affinity for ADP-actin than for ATP- or ADP-Pi-actin, and thus preferentially binds to ADP-actin subunits in the filaments. Cofilin enhances the depolymerization rate from the minus end by 20- to 30-fold, which is thought to result from a combination of destabilization of the terminal ADP-actin subunits and dissociation of them as cofilin–ADP-G-actin complexes [3]. The bound cofilin in the released complex prevents the exchange of nucleotide on ADP-G-actin, thus sequesters ADP-G-actin in the G-actin pool, which also contributes to the enhanced depolymerization rate. Binding of cofilin to F-actin also induces a twist in the double-helical structure and weakens the filaments, resulting in fragmentation (severing), which produces more filament ends where depolymerization occurs (Fig. 1). Dissociation of cofilin

Abbreviations: F-actin, actin filaments; G-actin, monomeric globular actin; G-site, G-actin-binding site; TnCl, taurine chloramine; Pi, phosphate; ADF, actin-depolymerizing factor; F-site, F-actin-binding site; HOCl, hypochlorous acid; MetO, methionine sulfoxide; DM, Cys139Ala/Cys147Ala double Cys mutant of cofilin; ABB, actin-binding buffer; Msr, methionine sulfoxide reductase; LIMK1, LIM domain kinase 1.

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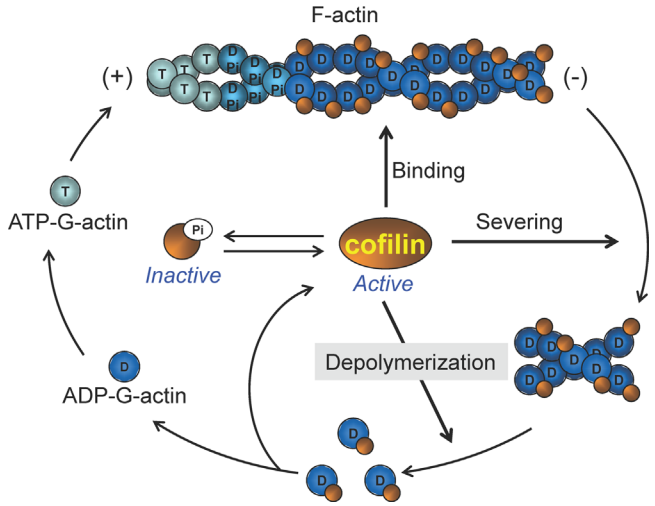

Fig. 1. Regulation of actin polymerization and depolymerization by cofilin. Under physiological salt conditions, monomeric ATP-G-actin can spontaneously polymerize into F-actin through the (+) end, ATP undergoes hydrolysis to release phosphate (Pi), and then ADP-G-actin depolymerizes at the (–) end. Cofilin has much higher affinity for ADP-actin than for ATP- or ADP-Pi-actin, and thus preferentially binds to ADP-actin subunits in the filaments. Cofilin enhances the depolymerization rate by 20- to 30-fold from a combination of destabilization of the terminal ADP-actin subunits and dissociation of them as cofilin–ADP-G-actin complexes. The bound cofilin in the released complex prevents the exchange of nucleotide on ADP-G-actin, thus sequesters ADP-G-actin in the G-actin pool, which also contributes to the enhanced depolymerization rate. Binding of cofilin to F-actin also induces a twist in the double-helical structure, resulting in fragmentation (severing), which produces more filament ends where depolymerization occurs. Cofilin is inactivated through phosphorylation that dramatically lowers its affinity for both F-actin and ADP-G-actin. Dissociation of cofilin from cofilin–ADP-G-actin complexes is promoted by profiling protein, which accelerates the nucleotide exchange of ADP for ATP.

from released cofilin–ADP-G-actin complexes is promoted by profiling, which dramatically accelerates the nucleotide exchange of ADP for ATP. Cofilin is regulated by phosphorylation/dephosphorylation on a conserved Ser^3 residue. The phosphorylated cofilin has very low affinity for both F-actin and ADP-G-actin [1,2].

Binding of cofilin to F-actin is a cooperative interaction that involves two sites on cofilin: an F-actin-binding site (F-site) and a G-actin-binding site (G-site). Cofilin initially binds to F-actin through the F-site and induces a twist. This twist facilitates interaction between cofilin and a neighboring actin subunit through the G-site on the opposite surface of cofilin, which stabilizes the F-actin/cofilin complexes [3,4]. A functional G-site is important for the depolymerization activity of cofilin because it is the G-site-based interaction between the cofilin and a terminal ADP-actin subunit at the minus end of filaments that results in their release from the F-actin complex.

In addition to its well-known actin regulatory property, recent findings have suggested that cofilin plays diverse roles in cell biology, including apoptosis, phospholipid metabolism, and gene expression [1]. Among these findings, previous work in this laboratory has demonstrated that oxidization of cofilin by taurine chloramine (TnCl) causes it to translocate to the mitochondria and induces apoptosis [5,6]. TnCl is generated by neutrophil cells during inflammation through chlorination of abundant ß-amino acid taurine (cellular concentration ~25 mM) by hypochlorous acid (HOCl) [7,8]. Unlike H_2O_2 or HOCl, TnCl has a limited spectrum of oxidative reactivity, targeting mainly Cys and Met residues in proteins [9,10]. This laboratory has shown that TnCl causes cells to die almost exclusively through apoptosis [6], and that the apoptosis is mediated by oxidation of cofilin [5]. Human cofilin-1 contains four free Cys and three Met residues [4], all of which are susceptible to TnCl-mediated oxidation, resulting in the formation of intramolecular disulfide bonds and methionine sulfoxide (MetO), but only Cys oxidation causes cofilin to induce mitochondrial damage [5].

Oxidation of proteins can cause them to lose function. Oxidation may inhibit cofilin's ability to regulate actin dynamics, and reduced actin dynamics in cells has been associated with reduced mitochondrial membrane potential and increased sensitivity to apoptotic insult [11]. It is possible that altered actin dynamics due to functional impairment of oxidized cofilin may also play a role in TnCl-induced apoptosis. But the molecular effects of cofilin oxidation by TnCl on its F-actin-binding and depolymerizing activities have not been explored.

In this study, by using recombinant human cofilin-1 wild-type protein, Cys to Ala mutants of cofilin, and a commercially available rabbit skeletal muscle α-actin (Cytoskeleton Inc.) that shares 100% sequence homolog with human α-actin [12], we have shown that the oxidation of cofilin by TnCl inhibits its ability to bind with F-actin and to catalyze depolymerization of F-actin. This functional impairment is not caused by the oxidation of Cys, but by the oxidation of Met, mainly at the Met^{115} residue located in the G-actin-binding site. We have also found that at high concentrations, TnCl can directly depolymerize F-actin–cofilin complexes and induce the formation of actin–cofilin heterodimer through covalent, nondisulfide bonding.

Materials and methods

Expression and purification of wild-type and site-specific mutant cofilin

The coding regions for wild-type human cofilin-1 and for all the site-specific Cys to Ala mutants were obtained by PCR amplification of cDNA cloned in pCMV6-XL5 (Origene) using 5′ primer (5′-GCCGCCCCATATGGCCTCCGGTGTGGCTGTCTCTGATGGTGTCA-3′) and 3′ primer (5′-GGATCCGGATCCTCACAAAGGCTTGCCCTCCAGGGA-3′). Nde-I and BamHI sites were incorporated at the 5′ and 3′ ends of the cofilin-encoding region, respectively. The construction of mutants with Cys to Ala mutations was described previously [5]. The genes were inserted into the Nde-I and BamHI sites of a pET-17b vector (Novagen). The $Cys^{39}Ala/Cys^{80}Ala$ double mutant and the four Cys to Ala quadruple mutant were generated using the Stratagene QuickChange Multi Site-Directed Mutagenesis Kit (Agilent Cat. No. 200514) and C39F and C80F primers [5]. The parental plasmids were the pET-17b plasmids containing either the WT coding region or the double Cys mutant $Cys^{139}Ala/Cys^{147}Ala$ (DM) coding region.

All plasmids were transformed into BL21(DE3)pLysS competent cells (Stratagene). Recombinant proteins were purified from the supernatants of cell lysates after sonication in 50 mM sodium phosphate (pH 7.2) and 1 mM DTPA, supplemented with 1X protease inhibitor cocktail (Thermo). The supernatants were pretreated twice with 1% (w/v) streptomycin sulfate precipitation to remove nucleic acids [13]. Cofilin proteins were precipitated by 50–80% (saturation) ammonium sulfate cut, dissolved in Tris-EDTA (10 mM Tris, pH 8.0, 1 mM EDTA) buffer, and dialyzed against the same buffer overnight using Thermo Slide-A-Lyzer Dialysis Cassettes (10 kDa MWCO). DEAE-cellulose resin (Whatman) was resuspended into the dialyzed samples and mixed for 30 min with gentle rotation and then removed by centrifugation. Cofilin was in the supernatant. Final purification was performed on an Agilent 1260 Infinity HPLC system using Beckman UltraSpherogel SEC 2000 column of 7.5 mm × 30 cm in the same Tris-EDTA buffer. The purified proteins were concentrated to 2 mg/mL with Millipore Amicon Ultra Centrifugal Filters (10 kDa MWCO), quick-frozen on dry ice, and stored at −80 °C. Cofilin concentration was determined