Communication

pH Control of Actin Polymerization by Cofilin*

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Cofilin, a 21,000 molecular weight actin-regulatory protein (Nishida, E., Maekawa, S., and Sakai, H. (1984) Biochemistry 23, 5307–5313), was here shown to be capable of reversibly controlling actin polymerization and depolymerization in a pH-sensitive manner. When cofilin was reacted with F-actin at different pH, the depolymerized actin concentration (≈ monomeric actin concentration) was higher at elevated pH. At pH < 7.3, the monomeric actin concentrations did not exceed 1 μM even in the presence of excess amounts of cofilin, whereas at pH > 7.3 it increased in proportion to the concentration of cofilin added, and complete depolymerization of F-actin occurred by the addition of an excess amount of cofilin. Moreover, in the presence of cofilin, rapid interconversion of monomeric and polymeric forms of actin can be induced by simply changing the pH of the medium. Thus, this study provides a new possible mechanism regulating actin polymerization, pH control.

Actin is a major constituent of cytoskeletons in mammalian cells, and rapid changes in polymeric states of actin occur during cell cycle and morphological changes of cells. In cultured cells, transient disruption of actin-containing stress fibers occurs in response to stimulation by growth factors and tumor promoters (1–5). It is widely believed that rapid interconversion of monomeric and polymeric forms of actin in cells is controlled by one or more actin-binding proteins (6–8). Actually, several proteins have been reported to be capable of rapidly depolymerizing actin filaments (6–12). However, a physiologically possible control mechanism for their activities has not been established, although the action of mammalian actin-depolymerizing protein (destrin) can be reversed by raising the ionic strength (12).

Cofilin is a 21,000 molecular weight actin-binding protein (13), which was purified from mammalian brain (14) and kidney (15). Our preliminary experiment with polyclonal antibodies against porcine brain cofilin indicated the existence of cofilin in a wide variety of different mammalian cell types.1 A previous study revealed that at near neutral pH cofilin binds to F-actin in a 1:1 molar ratio of cofilin to actin monomer in the filament, shortens the average length of the filament, and increases the steady state concentration of monomeric actin to a limited extent (13). Cofilin was also found to be able to bind to monomeric actin in a 1:1 molar ratio with an apparent dissociation constant of 0.1–0.2 μM at pH 7.1 (16). Moreover, the binding of cofilin to F-actin blocks the binding of tropomyosin to F-actin and inhibits actin-myosin interactions (13). In the course of the effort to elucidate a control mechanism for the interaction of cofilin with actin, we found that changes in pH greatly affect the action of cofilin on actin. We report, here, that cofilin is capable of reversibly controlling actin polymerization and depolymerization in a pH-sensitive manner.

MATERIALS AND METHODS

Rabbit skeletal muscle actin was prepared by the method of Spudich and Watt (17) and further purified by gel filtration on Sephadex G-100 equilibrated with a buffer solution containing 0.1 mM CaCl2, 0.2 mM ATP, 0.1 mM DTT, 0.03% NaN3, and 2 mM HEPES, pH 7.8. Actin concentration was determined by UV absorption measurement based on A280 = 6.5. Cofilin was purified from crude extracts of porcine brains by a new method that involved ammonium sulfate fractionation and sequential chromatography on a hydrophobic column, hydroxypatite, phosphocellulose, and Sephadex G-75. The purified cofilin was homogeneous, and the mode of its interaction with actin was almost identical to what had been reported previously using DNase I column-purified cofilin. The detailed description of this new purification procedure will be described elsewhere.2 The concentration of monomeric actin was determined by the DNase I inhibition assay as described by Blikstad et al. (18). The concentration of nonsedimented actin was assayed by centrifugation followed by SDS-polyacrylamide gel electrophoresis. An aliquot (0.15 ml) of sample solution was centrifuged at 100,000 × g for 40 min, and the resulting supernatant and pellet fractions were electrophoresed on SDS-polyacrylamide gels. Gels were stained with Coomassie Blue, and the intensity of the actin band was determined by scanning the gels with a densitometer. SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (19).

RESULTS AND DISCUSSION

When F-actin was reacted with about an equimolar concentration of cofilin, partial depolymerization of F-actin was induced and the concentration of monomeric actin was increased over a wide range of pH (6.3–8.3). The increase in the monomeric actin concentration was nearly constant between pH 6.3 and 7.3, while at pH > 7.3 it was increased with elevating pH (Fig. 1). A similar pH profile was obtained by both the DNase I inhibition assay (Fig. 1, O and □) and the sedimentation assay (Fig. 1, △ and ■). Since in the absence of cofilin the monomeric actin concentration did not markedly vary over the pH range shown in Fig. 1, the above results indicate that the ability of cofilin to depolymerize F-actin is stronger at alkaline pH.

To clarify this point in more detail, the ability of cofilin to depolymerize F-actin at pH 8.3 was compared with that at pH 7.0 (Fig. 2). When increasing concentrations of cofilin

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2) The abbreviations used are: DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis(2-aminoethyl ether)-N,N′N,N′-tetraacetic acid; PIPES, piperazine-N,N′-bis(2-ethanesulfonic acid); MFS, 2-(N-morpholino)ethanesulfonic acid.

3) N. Yonezawa, E. Nishida, S. Maekawa, and H. Sakai, manuscript in preparation.

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were reacted with F-actin at pH 7.0, the depolymerized actin concentration increased slightly, but reached a constant plateau level; i.e. complete depolymerization of F-actin cannot be induced even in the presence of excess amounts of cofilin (Fig. 2, closed symbols), as was previously shown at pH 7.3 (13). In contrast, at pH 8.3 the depolymerized actin concentration increased in proportion to the concentration of cofilin added, and complete depolymerization occurred by the addition of an excess amount of cofilin (molar ratio of cofilin to actin of 2-2.5:1) (Fig. 2, open symbols). When this depolymerized actin/cofilin mixture (cofilin:actin = 2:1) was gel-filtered on a Sephadex G-100 in a medium of M&lz + KCl at pH 8.3, actin emerged at an elution position corresponding to an apparent molecular mass of 45-70 kDa with about a half of cofilin coeluting with actin (data not shown), indicating that the depolymerized actin exists as a monomer in the form of a complex with cofilin.

To examine whether changes in Ca\(^{2+}\) concentration affect the pH-dependent action of cofilin, the ability of cofilin to depolymerize F-actin was investigated in the presence (0.1 mM CaCl\(_2\)) and absence of Ca\(^{2+}\) (1 mM EGTA) both at pH 7.0 and 8.3. The result shown in Table I clearly indicates that the presence or absence of Ca\(^{2+}\) does not affect the pH-dependent actin-depolymerizing action of cofilin, i.e. the depolymerizing activity is much stronger at pH 8.3 than at pH 7.0 irrespective of the Ca\(^{2+}\) concentration, although the depolymerized actin concentration is slightly higher in the presence of Ca\(^{2+}\) than in the absence of Ca\(^{2+}\).

Whether the pH-dependent action of cofilin is reversible, or not, appeared to be a key point, when one considers the possible physiological significance of this phenomenon. Fig. 3a shows that polymerization and depolymerization of actin can be reversibly controlled in the presence of cofilin by simply changing the pH of the medium. When F-actin was mixed with cofilin (cofilin:actin = 2.2:1) at pH 8.2, complete depolymerization occurred. Next, the pH of this mixture was lowered to 6.6 (Fig. 3a, first arrow). This shift in pH induced a rapid polymerization of actin. After this polymerization of actin reached a plateau, the pH was then raised to 8.0 (Fig. 3a, second arrow). This induced a depolymerization of F-actin, which was very fast.

The time course of cofilin-induced depolymerization of F-actin was investigated in more detail (Fig. 3b). When cofilin was reacted with F-actin at pH 7.0 (cofilin:actin = about 2:1), a partial depolymerization occurred. The resultant F-actin binds cofilin along its entire length and has a shorter average length (13). Then, the rise in pH (to 8.2) induced a very rapid and complete depolymerization of the F-actin (Fig. 3b). The reaction was completed within 3 min. Interestingly, when the mixing of cofilin with F-actin was performed at pH 8.2 from

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**Table I**

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<thead>
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<th>Cofilin / Actin (Molar ratio)</th>
<th>% F-actin</th>
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<tr>
<td>0</td>
<td>100</td>
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<tr>
<td>1</td>
<td>50</td>
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**Fig. 1. Effect of varying pH on the concentration of monomeric actin (O and ◦) or non sedimented actin (Δ and △) in the presence (closed symbols) or absence (open symbols) of cofilin.** F-actin was prepared by polymerizing purified muscle actin in 2 mM MgCl\(_2\). Then, the F-actin was mixed with about an equimolar concentration of nonsedimented actin was determined as described under "Materials and Methods" using an aliquot (0.15 ml) of each mixture (O and ◦). As a control, the monomeric or nonsedimented actin concentration in the absence of cofilin was also determined.

**Fig. 2. Effect of increasing concentrations of cofilin on F-actin at pH 7.0 (O and △) or pH 8.3 (O and ◦).** Increasing concentrations of cofilin were reacted with F-actin at 22 °C for about 1 h at pH 7.0 or 8.3, and the concentration of monomeric actin was determined by the DNase I inhibition assay. The data are expressed as per cent F-actin concentration. The buffer solution contained 2 mM MgCl\(_2\), 70 mM KCl, and 50 mM PIPES, pH 7.0 (O and △) or 50 mM HEPES, pH 8.3 (O and ◦). The total actin concentrations were 4.1 µM (O and ◦) and 3.4 µM (Δ and △).
*pH Control of Actin Polymerization*

![Diagram](image_url)

**Fig. 3.** a. Reversible polymerization and depolymerization of actin with cofilin depending on pH of the medium. F-actin was completely depolymerized by the addition of cofilin (cofilin:actin = 2:1) at pH 8.2 and 22 °C in a solution consisting of 2 mM MgCl₂, 70 mM KCl, and 10 mM Tris, pH 8.2. At zero time, the pH of the mixture was lowered to 6.0 by the addition of the concentrated MES buffer (final 40 mM MES and 9 mM Tris, first arrow). Depolymerization of actin occurred. After incubation at 22 °C for 40 min, the pH was then adjusted to 8.0 by the addition of the concentrated Tris buffer (final 90 mM Tris and 30 mM MES, second arrow). Depolymerization of actin was induced. At each time the concentration of monomeric actin was determined by the DNase I inhibition assay. Total actin concentration was changed from 3.45 μM (at pH 8.2) to 3.17 μM (at pH 6.6) and 2.64 μM (at pH 8.0) and shown by broken lines. b. Kinetics of cofilin-induced depolymerization of actin. F-actin (3.65 μM) was reacted with cofilin (7.53 μM) at 22 °C in a solution consisting of 2 mM MgCl₂, 70 mM KCl, and 15 mM PIPES, pH 7.0 (at zero time). The concentration of monomeric actin increased gradually and reached a plateau. Twenty-eight minutes after incubation, the pH was shifted to 8.2 by the addition of the concentrated Tris buffer (final 45 mM Tris and 14 mM PIPES). Total actin concentration was reduced to 3.39 μM. A rapid and complete depolymerization occurred. The inset shows the time course of actin depolymerization which was induced by mixing F-actin with cofilin at pH 8.2 from the beginning in a solution consisting of 2 mM MgCl₂, 70 mM KCl, and 60 mM Tris. The protein concentrations were 3.44 μM actin and 7.48 μM cofilin. In this case, the depolymerization was relatively slow. The concentration of monomeric actin was determined by the DNase I inhibition assay.

At the beginning, the depolymerization was relatively slow. For the completion of the reaction, 10 to 20 min was required (Fig. 3b, inset). We speculate that the previous binding of cofilin to F-actin and/or the shorter average length of the F-actin might facilitate the depolymerization in the former type of experiment (i.e., pH shift).

In this study, cofilin was found to be capable of reversibly controlling actin polymerization and depolymerization in a pH-sensitive manner. This paper may be the first to demonstrate a pH-dependent control of actin polymerization and depolymerization, although it has been shown that some actin-binding proteins such as Dictyostelium discoideum 95-kDa protein (20) and vertebrate brain microtubule-associated proteins (21) cross-link F-actin in a pH-dependent manner.

There is evidence that cytoplasmic pH may have an important role in a variety of biological systems (22). Recently, it has been demonstrated that a rise in pH occurs in quiescent cells stimulated by growth factors and tumor promoters (23–25), and this alkalization seems to be necessary for the initiation of DNA synthesis (26). On the other hand, it is widely known that such treatments of quiescent cells induce a rapid disorganization of actin-containing structures (1–5). Thus, it is interesting to speculate that the rise in pH induces disorganization of actin through the action of cofilin.

A number of actin-binding proteins have been found and characterized (6–8). Since the activities of many of them are regulated by changes in Ca²⁺ concentration, much attention has been paid to the Ca²⁺ control of actin filament organization (6–8). This study provides another possibility that changes in intracellular pH regulate interconversion of polymeric and monomeric forms of actin in cells.

**REFERENCES**