Studies on the interaction between actin and cofilin purified by a new method

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Cofilin is a 21 000- M_r actin-binding protein that widely exists in mammalian tissues. (1) A new purification procedure for porcine brain cofilin has been developed that involves $(\text{NH}_4)_2\text{SO}_4$ fractionation and sequential chromatographies on Toyo Pearl and butyl-Toyo Pearl hydrophobic columns, hydroxyapatite, phosphocellulose and Sephadex G-75 gel-filtration columns. The purified cofilin bound to F-actin and increased the amount of G-actin to a limited extent, as previously reported [Nishida, Maekawa & Sakai (1984) *Biochemistry* 23, 5307–5313]. (2) The binding of cofilin to F-actin was scarcely affected by Mg^{2+} , Ca^{2+} or by calmodulin. However, the binding was diminished by increasing concentrations of KCl, but was only slightly affected by temperature. (3) Cofilin and either α -actinin or filamin could bind to F-actin simultaneously with some competition, but the binding of caldesmon to F-actin was markedly inhibited by cofilin. Phalloidin inhibited the binding of cofilin to F-actin, and protected F-actin from depolymerization by cofilin.

INTRODUCTION

To elucidate the mechanisms which regulate the polymerization state of actin, many actin-binding proteins have been purified from various types of cells and characterized (Korn, 1982; Weeds, 1982; Pollard & Cooper, 1986). Cofilin (Nishida *et al.*, 1984) is a 21 000- M_r actin-binding protein that has been purified from porcine brain (Maekawa *et al.*, 1984) and porcine kidney (Muneyuki *et al.*, 1985) by using a DNAase I affinity column. It was shown that cofilin has the ability to bind to both G and F-actin under physiological conditions (Nishida *et al.*, 1984). Cofilin not only coats the actin filaments with a 1:1 molar ratio of binding to actin, but also binds to G-actin in the same 1:1 molar ratio (Nishida *et al.*, 1984; Nishida, 1985). We have also found that cofilin can control actin polymerization and depolymerization reversibly in a pH-sensitive manner (Yonezawa *et al.*, 1985). Moreover, immunological studies have shown that cofilin is present in a wide variety of mammalian tissues and cells (Yonezawa *et al.*, 1987) and that cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells (Nishida *et al.*, 1987).

Many actin-binding proteins appear to react with actin filaments in non-muscle cells. Therefore it seemed necessary to examine the interactions between actin filaments and cofilin and other F-actin-binding proteins. It has previously been shown that the binding of cofilin to F-actin inhibits the binding of tropomyosin to F-actin as well as actin-myosin interactions (Nishida *et al.*, 1984).

In the present paper we report a new purification procedure for cofilin that omits the DNAase I affinity column chromatography and describe the effects of Mg^{2+} , KCl and Ca^{2+} , as well as those of calmodulin and temperature, on the binding of cofilin to F-actin. The present paper also deals with the effects of α -actinin, filamin and caldesmon, as representative F-actin-

binding proteins, on the interaction between cofilin and actin measured by the pelleting assay. In addition, we also examined the effect of phalloidin on the binding of cofilin to actin filaments. Phalloidin is known to bind to F-actin tightly and stabilize it (Lengsfeld *et al.*, 1974; Dancker *et al.*, 1975; Coluccio & Tilney, 1984).

MATERIALS AND METHODS

Purification of cofilin

All steps were carried out at 0–4 °C. About 500 g of porcine brains (Tokyo Shibaura Zoki Co., Tokyo, Japan) were homogenized with a mixer in 300 ml of 10 mM-Tris/1 mM-EGTA/1 mM-ATP/1 mM-DTT/1 mM-PMSF, pH 7.5, and then centrifuged at 20 000 g for 1 h. $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to 50% saturation. After stirring for 1 h, the mixture was centrifuged at 20 000 g for 20 min. The supernatant was applied to a Toyo Pearl HW 65C (Toyo Soda Manufacturing Co., Tokyo, Japan) hydrophobic column (25 mm $\times$ 85 mm) equilibrated with buffer A (10 mM-Tris/1 mM-EGTA/0.5 mM-DTT, pH 7.5) containing 50%-satd. $(\text{NH}_4)_2\text{SO}_4$, the column then being washed with 120 ml of the same buffer. The flow-through fractions were directly applied to a butyl Toyo Pearl 650C (Toyo Soda Manufacturing Co.) hydrophobic column (18 mm $\times$ 240 mm) equilibrated with buffer A containing 50%-satd. $(\text{NH}_4)_2\text{SO}_4$. After washing the column with the same solution, the adsorbed proteins were eluted with a linear gradient of 50–0%-satd. $(\text{NH}_4)_2\text{SO}_4$ in buffer A (400 ml). After checking by means of SDS/polyacrylamide-gel electrophoresis, fractions containing cofilin were collected and $(\text{NH}_4)_2\text{SO}_4$ was added to 90% saturation, followed by centrifugation at 20 000 g for 20 min. The pellet was suspended in about 8 ml of 10 mM- KH_2PO_4 /0.1 M-NaCl/0.1 mM-DTT, pH 6.7, dialysed overnight against the same solution, and then chromatographed on a Bio-Gel HTP (Bio-Rad

Laboratories, Richmond, CA, U.S.A.) hydroxyapatite column (15 mm × 80 mm), a linear gradient of 10–150 mM-KH₂PO₄ (160 ml) being used for elution. Fractions containing cofilin were collected and dialysed overnight against a buffer solution containing 10 mM-Mes and 0.1 mM-DTT, pH 6.4. The dialysed fraction was chromatographed on a phosphocellulose (P11; Whatman International, Maidstone, Kent, U.K.) column (14 mm × 28 mm), a linear gradient of 0–300 mM-NaCl in the same buffer solution being used as eluent. Fractions containing cofilin were combined and concentrated to a volume of about 2 ml by using a YM-05 (Amicon Co., Lexington, MA, U.S.A.) membrane. The concentrated sample was gel-filtered on a Sephadex G-75 (Pharmacia Co., Uppsala, Sweden) column (13 mm × 780 mm) equilibrated with 10 mM-Pipes/0.1 M-KCl/0.1 mM-DTT, pH 7.3, as a final step. The concentration of cofilin was estimated by measuring the absorbance at 280 nm, an $A_{280}^{1\%}$ value of 8.7 being assumed.

Purification of other proteins

Rabbit skeletal-muscle actin was prepared by the method of Spudich & Watt (1971) and further purified by gel filtration on Sephadex G-100 (Pharmacia) equilibrated with a buffer solution containing 0.1 mM-CaCl₂, 0.2 mM-ATP, 0.1 mM-DTT, 0.01% NaN₃ and 2 mM-Hepes, pH 7.8. Actin concentration was determined by measurement of u.v. absorption, an $A_{290}^{1\%}$ value of 6.5 being assumed. Calmodulin was purified from porcine brain as described previously (Nishida *et al.*, 1979). Filamin and α -actinin were purified from bovine stomach smooth muscle as described by Feramisco & Burridge (1980). Caldesmon was purified from bovine stomach smooth muscle by the method of Bretscher (1984). The concentrations of filamin, α -actinin and caldesmon were determined by measuring the absorbance at 280 nm by using $A_{280}^{1\%}$ of 6.8 (Wang, 1977), 9.7 (Suzuki *et al.*, 1976) and 3.0 (Bretscher, 1984) respectively.

Pelleting assay

The binding of proteins to F-actin was examined by the pelleting assay. All protein solutions except that of actin were dialysed overnight against a buffer solution containing 10 mM-Pipes, 0.1 M-KCl and 0.1 mM-DTT, pH 7.3, and centrifuged at 100 000 *g* for 40 min before use. G-actin was polymerized before the assay by the addition of 2 mM-MgCl₂ or 60 mM-KCl. Protein solutions were mixed in centrifuge tubes and incubated at about 22 °C for 20 min, unless noted otherwise. The standard buffer solution consisted of 2 mM-Mg²⁺, 80 mM-KCl, 9 mM-Pipes and 0.09 mM-DTT, pH 7.3, in a total volume of 150 μ l. Protein concentrations are given in the Figure legends. After centrifugation at 100 000 *g* for 40 min, the supernatants were carefully removed and the pellets were suspended in 150 μ l of 1% (w/v) SDS solution. Equal volumes of the supernatant and pellet were electrophoresed on SDS/polyacrylamide gels. Since the staining intensities of proteins were linear in the range of 0.3–3 μ g/lane in our SDS/polyacrylamide-gel-electrophoretic experiments, we adjusted the sample volume of pellet or supernatant fractions electrophoresed in one lane to the limit of this range. After staining and destaining the gels, staining intensities of both the pellet and supernatant fractions were determined with a densitometer (Beckman Instruments, Fullerton, CA,

U.S.A.), and the amount of each protein in pellet or supernatant was determined by using the equation:

$$\text{Amount of each protein} = \frac{\text{Staining intensity of pellet or supernatant}}{\text{Staining intensity of pellet plus that of the supernatant}} \times \text{Amount of each protein in the reaction solution}$$

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed on 13–14%-(w/v)-polyacrylamide separating gels as described by Laemmli (1970).

RESULTS AND DISCUSSION

Purification of cofilin by a new procedure

Cofilin was previously purified by using DNAase I affinity chromatography (Nishida *et al.*, 1984; Mackawa *et al.*, 1984; Muneyuki *et al.*, 1985). Because the purification depends on the ability of cofilin to bind to the DNAase I column through actin, proteins that do not bind to G-actin could not be purified by this affinity column chromatography. However, there might be a species of cofilin that cannot bind to G-actin or has only a low affinity for G-actin. Therefore we have developed a new purification procedure that omits the DNAase I affinity-chromatography step.

Fig. 1 shows elution profiles of proteins at each chromatography step for cofilin purification, the protein composition at each step being given in Fig. 2. Cofilin was scarcely adsorbed to the Toyo Pearl hydrophobic column under conditions of 50% saturation with (NH₄)₂SO₄. Cofilin was eluted from the butyl Toyo Pearl column in a range of 20–5% saturation of (NH₄)₂SO₄ (Fig. 1a). On the hydroxyapatite column cofilin was eluted at about 70 mM-KH₂PO₄ with the first protein peak (Fig. 1b and Fig. 2, lane 5). On phosphocellulose column chromatography, 50% of the cofilin was eluted with 120–150 mM-NaCl (Fig. 1c). The remaining cofilin was eluted with NaCl at higher concentrations (150–300 mM). These delayed fractions were not used, however, since contaminating proteins in these fractions were not completely removed by any subsequent column chromatography. Finally, cofilin was eluted as a symmetrical peak on Sephadex G-75 gel filtration (Fig. 1d). The final fraction gave a single band on SDS/polyacrylamide gels (Fig. 2, lanes 7 and 7'). The yield of cofilin was 0.5–1.5 mg from 500 g of porcine brain.

General features of interaction of the purified cofilin with actin were the same as those of cofilin purified by a previous method (see below). Cofilin that was partially purified from the fractions eluted at 150–300 mM-NaCl from the phosphocellulose column by using Sephadex G-75 gel filtration, also showed characteristics indistinguishable from that of cofilin purified by a previous method (results not shown). The merit of the new method is that whereas a DNAase I affinity column can be used only 5–6 times because of the inactivation of DNAase I, Toyo Pearl and butyl Toyo Pearl hydrophobic columns can be used many times, and the yield of cofilin is more constant than by the previous method.

Fig. 1. Elution profiles of proteins at each cofilin purification step

(a) Butyl Toyo Pearl hydrophobic column; (b) hydroxyapatite column; (c) phosphocellulose column; (d) Sephadex G-75 gel filtration. Absorbances at 280 nm of each fraction and the salt gradients are shown by the continuous and broken lines respectively. Elution positions of cofilin are indicated by horizontal bars (—).

Fig. 2. SDS/polyacrylamide-gel electrophoresis of cofilin fractions at each purification step

Lane 1, crude extract; lane 2, 50%-satd.-(NH₄)₂SO₄ supernatant; lane 3, flow-through fraction of Toyo Pearl hydrophobic column chromatography; lane 4, butyl Toyo Pearl hydrophobic column fraction; lane 5, hydroxyapatite column fraction; lane 6, phosphocellulose column fraction; lanes 7 and 7', Sephadex G-75 column fractions [2 μg (lane 7) and 6 μg (lane 7')].

General features of the interaction between cofilin and F-actin

When cofilin was absent, approx. 95% of actin was polymerized in the presence of Mg²⁺ at concentrations higher than 1 mM (Fig. 3a). In the presence of cofilin, however, the amount of F-actin was reduced by about

1 μM, and cofilin bound to F-actin in a nearly equimolar ratio. The binding of cofilin was not affected by changes in the Mg²⁺ concentration from 0 mM to 5 mM. However, at 8 mM-Mg²⁺ the binding of cofilin was a little diminished.

In Fig. 3(b) the effect of the KCl concentration on the binding of cofilin to F-actin is shown. In the absence of cofilin, the amount of F-actin slightly decreased with increasing KCl concentration. Addition of cofilin caused F-actin to decrease to a certain extent, but the remaining F-actin remained almost constant when the KCl concentration was increased up to 0.5 M. On the other hand, cofilin bound to F-actin was diminished as the concentration of KCl increased. At 500 mM-KCl, cofilin could not bind to F-actin at all.

It has previously been reported that the affinity of cofilin for G-actin is decreased at high KCl concentration (Nishida, 1985). The reduction of binding to F-actin at high ionic strength seems to be a character common to F-actin-binding proteins. For example, the binding of filamin (Wang & Singer, 1977) or caldesmon (Bretscher, 1984) to F-actin is also diminished at high salt concentrations. This suggests that these proteins bind to F-actin, at least in part, by electrostatic affinity. The cofilin-binding site of actin has been determined to be 1–12 amino acid residues from the N-terminus of actin (Muneyuki *et al.*, 1985). It has been suggested that the carboxy groups in this region of actin and the amino groups of cofilin interact with each other to contribute to the binding of cofilin to actin (Muneyuki *et al.*, 1985).

Table 1 shows that the binding of cofilin to F-actin was reduced only a little at 35 °C as compared with that at 0 °C, indicating that cofilin is capable of binding to F-actin at physiological temperature.

Several actin-binding proteins are known to be regulated by free Ca²⁺ *in vitro* (Mimura & Asano, 1979;

Fig. 3. Effect of Mg^{2+} (a) and KCl (b) concentration on the binding of cofilin to F-actin

In (a), F-actin ($3.3 \mu\text{M}$) with or without cofilin ($3.2 \mu\text{M}$) was incubated in the presence of various concentration of Mg^{2+} . The concentration of F-actin in the absence (O) or presence (●) of cofilin and the concentration of cofilin bound to F-actin (▲) were determined as described in the Materials and methods section. In (b), F-actin ($3.0 \mu\text{M}$) with or without cofilin ($2.6 \mu\text{M}$), which had been dialysed overnight against 10 mM-Pipes/0.1 mM-DTT, pH 7.3, to remove KCl , was incubated with 2 mM- Mg^{2+} and various concentrations of KCl . The concentration of F-actin in the absence (O) or presence (●) of cofilin and the concentration of cofilin bound to F-actin (▲) were determined.

Table 1. Effect of temperature, calmodulin or Ca^{2+} on the binding of cofilin to F-actin

To examine the effect of temperature, F-actin ($3.3 \mu\text{M}$) and cofilin ($3.2 \mu\text{M}$) were incubated and centrifuged at 0°C or 35°C . The effects of Ca^{2+} and calmodulin (CaM) were examined by mixing F-actin ($3.3 \mu\text{M}$) and cofilin ($2.9 \mu\text{M}$) with or without calmodulin ($6.0 \mu\text{M}$) in a buffer solution containing either 0.1 mM- Ca^{2+} or 1 mM-EGTA at 22°C .

Conditions	Concn. (μM) of sedimented:	
	Actin	Cofilin
Temperature		
0°C	2.2	1.9
35°C	2.1	1.6
Ca^{2+} and CaM		
0.1 mM- Ca^{2+}	2.2	2.2
1 mM-EGTA	2.1	1.9
0.1 mM- Ca^{2+} + CaM	2.1	2.1
1 mM-EGTA + CaM	2.3	2.3

Yin *et al.*, 1980; BurrIDGE & FERAMISCO, 1981). A calmodulin-dependent F-actin-binding protein has also been reported (Sobue *et al.*, 1981). Therefore it seemed worthwhile to examine the effect of Ca^{2+} and calmodulin. The results are shown in Table 1, in which the change in the amount of cofilin bound to F-actin upon addition of 1 mM-EGTA falls within the experimental error. It was concluded that the binding of cofilin to F-actin is not regulated by Ca^{2+} . Moreover, calmodulin had no effect on the amount of cofilin bound to F-actin in either the presence or absence of Ca^{2+} .

It has already been reported that cofilin can regulate actin polymerization and depolymerization reversibly in a pH-sensitive manner (Yonezawa *et al.*, 1985). Thus

changes in the ionic strength and pH are factors which affect interactions of cofilin with actin *in vitro*.

Effect of α -actinin, filamin or caldesmon on the binding of cofilin to F-actin

We examined the effect of α -actinin, filamin or caldesmon on the interaction of cofilin with actin by the pelleting assay. In the first type of experiments, about an equimolar (with actin) amount of cofilin was mixed with F-actin, and then various amounts of α -actinin, filamin or caldesmon were added. As shown in Fig. 4, cofilin was not dissociated from F-actin by the addition of α -actinin, filamin or caldesmon. However, the amount of α -actinin or filamin bound to F-actin in the presence of cofilin reached nearly the same level as in the absence of cofilin. These suggest that cofilin, as well as either α -actinin or filamin, can bind to F-actin simultaneously. On the other hand, the binding of caldesmon to F-actin was markedly inhibited by cofilin. Caldesmon bound to F-actin only up to 40% saturation, even if very high concentrations of caldesmon were added (Fig. 5c).

Conversely, when a saturable amount of α -actinin, filamin or caldesmon was incubated with F-actin before the addition of cofilin, the binding of cofilin to F-actin was markedly inhibited by α -actinin or filamin, especially when low concentrations of cofilin were present (Figs. 5a and 5b, Δ , $\blacktriangle$). But when about an equimolar (with actin) amount of cofilin was added, cofilin could bind to F-actin at a level of 60–80% as compared with the amount bound in the absence of α -actinin or filamin, resulting in dissociation of the previously bound α -actinin or filamin partially or wholly respectively. Caldesmon did not inhibit the binding of cofilin at all and was dissociated from F-actin by the binding of cofilin to F-actin (Fig. 5c).

It should be noted that the changes in the amount of F-actin as a function of the cofilin concentration were the same irrespective of the presence of α -actinin, filamin or caldesmon (Fig. 5). This suggests that the inhibition of

Fig. 4. Effect of cofilin on the binding of α -actinin, filamin or caldesmon to F-actin

Cofilin ($3.2 \mu\text{M}$) was incubated for 20 min with F-actin ($3.0 \mu\text{M}$), then various amounts of α -actinin (0, 1.1, 2.1 or $3.5 \mu\text{M}$ in *a*), filamin (0, 0.21, 0.42, or $0.69 \mu\text{M}$ in *b*) or caldesmon (0, 0.44, 0.88 or $1.4 \mu\text{M}$ in *c*) were added. The concentrations of F-actin (●), cofilin bound to F-actin (▲) and α -actinin, filamin or caldesmon bound to F-actin (■) were plotted as a function of the concentration of α -actinin (*a*), filamin (*b*) or caldesmon (*c*). As a control, α -actinin ($3.5 \mu\text{M}$), filamin ($0.69 \mu\text{M}$) or caldesmon ($1.4 \mu\text{M}$) was allowed to react with F-actin ($1.9 \mu\text{M}$) in the absence of cofilin, and the concentration of α -actinin (*a*), filamin (*b*) or caldesmon (*c*) bound to F-actin was determined (□).

binding of cofilin to F-actin by α -actinin or filamin does not seem to affect the ability of cofilin to depolymerize F-actin. The mechanism of actin depolymerization by cofilin is not yet known, but it is likely that cofilin can depolymerize F-actin partially by sequestering free actin monomers as well as by interacting directly with F-actin protomers, because cofilin can bind to both G- and F-

Fig. 5. Effect of α -actinin, filamin and caldesmon on the binding of cofilin to F-actin

F-actin ($3.5 \mu\text{M}$) was first incubated for 15 min with $2.3 \mu\text{M}$ - α -actinin (*a*), $0.85 \mu\text{M}$ -filamin (*b*) or $1.3 \mu\text{M}$ -caldesmon (*c*), and then further incubated with 0, 1.3, 2.6 or $4.0 \mu\text{M}$ -cofilin for 20 min. The concentrations of F-actin (●) and cofilin bound to F-actin (▲) in the presence of α -actinin (*a*), filamin (*b*) or caldesmon (*c*) are shown as a function of the concentration of cofilin. As a control, the concentrations of F-actin (○) and cofilin bound to F-actin (△) in the absence of α -actinin (*a*), filamin (*b*) or caldesmon (*c*) are shown. The concentration of α -actinin (*a*), filamin (*b*) or caldesmon (*c*) bound to F-actin is represented by ■.

actin (Nishida *et al.*, 1984). Therefore, even if cofilin cannot attack F-actin directly because of previous binding of α -actinin or filamin to F-actin, cofilin could depolymerize F-actin by sequestering free G-actin. However, it is also possible that cofilin interacts directly with F-actin protomers that do not bind α -actinin or filamin directly, taking actin molecules away from F-actin and inducing the cutting and partial

Fig. 6. Marked inhibition by phalloidin of the cofilin binding to F-actin

F-actin ($3.0 \mu\text{M}$) was mixed with phalloidin (Sigma Chemical Co., St. Louis, MO, U.S.A.) ($10 \mu\text{M}$), and then various amounts of cofilin [0, 0.4, 0.8, 1.6, 2.4 or $3.2 \mu\text{M}$ at pH 7.3 and 0, 0.6, 1.2, 2.4 or $3.2 \mu\text{M}$ at pH 8.3 (20 mM-Tris/2 mM- MgCl_2 /70 mM-KCl)] were added. The amounts of F-actin ($\circ$, $\bullet$) and cofilin bound to F-actin (Δ , $\blacktriangle$) were determined as described in the Materials and methods section. Open and closed symbols refer to the data obtained at pH 8.3 and 7.3 respectively.

depolymerization of F-actin. The inhibition of binding of cofilin to F-actin by α -actinin or filamin might be due to steric hindrance or to the inability of cofilin to penetrate into the actin gel made of F-actin and either α -actinin or filamin. Irrespective of the reason for the inhibition of binding of cofilin to F-actin, it is reasonable, considering the above points, to assume that cofilin induces partial depolymerization of α -actinin or filamin containing F-actin.

In summary, the data shown in Figs. 4 and 5 indicate that cofilin and either α -actinin or filamin can bind to F-actin simultaneously, although the binding of cofilin to F-actin is affected by the prior binding of α -actinin or filamin. Caldesmon and cofilin compete with each other for binding to F-actin. Cofilin can depolymerize F-actin partially irrespective of the presence of α -actinin, filamin or caldesmon.

Effect of phalloidin

When phalloidin was first made to react with F-actin before the addition of cofilin, the binding of cofilin to F-actin was remarkably inhibited and F-actin was not depolymerized by cofilin (Fig. 6). We have shown that the ability of cofilin to depolymerize F-actin is stronger at higher pH (Yonezawa *et al.*, 1985). More than 80% of F-actin ($3 \mu\text{M}$) was depolymerized by cofilin ($6.3 \mu\text{M}$) in the absence of phalloidin at pH 8.3 (results not shown), whereas, in the presence of phalloidin, depolymerization by cofilin was not induced at all (Fig. 6). The results suggest that phalloidin blocks the interaction of cofilin with F-actin. Interestingly, we have recently found that cofilin is a component of actin rods induced in cultured fibroblastic cells treated with specific salt solutions,

dimethyl sulphoxide or heat, and that phalloidin derivatives do not bind to these actin rods. This is consistent with a result *in vitro* that cofilin-associated F-actin did not bind phalloidin (Nishida *et al.*, 1987).

One of possible explanations for the mutual interference in binding between cofilin and phalloidin is that the conformational change of actin molecule is induced by the binding of phalloidin (Wieland *et al.*, 1975; Harwell *et al.*, 1980) or cofilin (Nishida *et al.*, 1984). Another possibility is that the binding sites of cofilin and phalloidin are adjacent on the surface of the actin molecule although they are far apart from each other in the actin primary sequence. The binding site of phalloidin in the actin sequence was determined to be residues 117–119 and 355 (Vandekerckhove *et al.*, 1985), and cofilin has been shown to cross-link to residues 1–12 of actin (Muneyuki *et al.*, 1985). A low- M_r actin-binding protein, depactin, has been reported to cross-link to residues 1–12 and 357–375 of actin (Sutoh & Mabuchi, 1986), so the *N*-terminal region and *C*-terminal region of actin may not be far apart in the actin molecule. Thus it is possible that the cofilin-binding site is located near the *C*-terminal (residue 355) phalloidin-binding site. Furthermore, it has been reported that the nucleotide-binding site of actin is adjacent to residues 117–119, and phalloidin blocks the exchange of nucleotide (Barden *et al.*, 1987). Interestingly, cofilin has been shown to decrease the rate of exchange of actin-bound ATP (Nishida, 1985). These facts are also consistent with the idea that the binding sites of cofilin and phalloidin are close to each other.

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