A Short Sequence Responsible for Both Phosphoinositide Binding and Actin Binding Activities of Cofilin*

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Naoto Yonezawa, Yoshimi Homma, Ichiro Yahara, Hikoichi Sakai, and Eisuke Nishida

From the Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan, the Department of Biosignal Research, Tokyo Metropolitan Institute of Gerontology, Itabashi, Tokyo 173, Japan, and the Department of Cell Biology, The Tokyo Metropolitan Institute of Medical Science, Honkomagome, Tokyo 113, Japan

Cofilin is a widely distributed actin-modulating protein that has abilities to bind along the side of F-actin and to depolymerize F-actin. Both abilities of cofolin can be inhibited by phosphoinositides such as phosphatidylinositol, phosphatidylinositol 4-monophosphate, and phosphatidylinositol 4,5-bisphosphate (PIP2). We have previously shown that the synthetic dodecapeptide corresponding to Trp104-Met115 of cofolin is a potent inhibitor of actin polymerization (Yonezawa, N., Nishida, E., Iida, K., Kumagai, H., Yahara, I., and Sakai, H. (1991) J. Biol. Chem. 266, 10485–10489). In this study, we have found that the inhibitory effect of the synthetic dodecapeptide on actin polymerization is canceled specifically by phosphatidylinositol, phosphatidylinositol 4-monophosphate and PIP2. We further show that the dodecapeptide as well as cofolin binds to PIP2 molecules and inhibits PIP2 hydrolysis by phospholipase C. Thus, the actin-binding dodecapeptide sequence of cofolin may constitute a multifunctional domain in cofolin.

Cofilin (1) is a widely distributed actin-binding protein with an apparent molecular mass of 21 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cofilin binds not only along F-actin but also to G-actin and depolymerizes F-actin in a pH-dependent manner in vitro (1–3). Cofilin forms intranuclear and/or cytoplasmic actin/cofilin rods in cultured cells in response to various stimuli (4).

Cofilin comprises 166 amino acid residues as revealed by the analyses using cDNA cloning and sequencing (5). Our recent study has shown that Lys112 and/or Lys114 of cofolin can be cross-linked by a chemical zero length cross-linker to actin molecule and that the synthetic dodecapeptide, WAPECAPLKSKM, patterned on the sequence around the cross-linking site (corresponding to Trp104-Met115) of cofolin is a potent inhibitor of actin polymerization (6).

It has been reported that polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 4-monophosphate (PIP) inhibit the actin-modulating activities of profilin, gelsolin, and villin in vitro (7–10). Polyphosphoinositides induce the dissociation of profilactin and gelsolin-actin complex by interacting with profilin and gelsolin, respectively. In addition, we reported that cofolin also has the polyphosphoinositides sensitivity (11). In the case of cofolin, not only PIP and PIP2 but also phosphatidylinositol (PI) inhibited the interactions of cofolin with both G- and F-actin.

Previous studies using gelsolin fragments generated by the limited proteolysis or the gene truncation of plasma gelsolin cDNA revealed that the PIP2-binding site on gelsolin sequence is on residues 150–160 (12, 13). It is also suggested that the sequence of residues 150–160 may be the F-actin-binding site (12, 13). Cofilin does not have the sequence similar to this polyphosphoinositide binding sequence of gelsolin. The polyphosphoinositide binding sequences of other actin-binding proteins have not yet been identified. Here, we have shown that the actin binding sequence of cofolin (Trp104-Met115), previously identified by the chemical cross-linking study and by the use of the synthetic peptide, is a phosphoinositide binding sequence. Thus, inhibition of actin polymerization by the synthetic dodecapeptide corresponding to Trp104-Met115 of cofolin is canceled specifically by phosphoinositides such as PIP3, PIP2, and PI.

Recent reports have revealed that profilin binds with high affinity to PIP2 molecules (7, 14) and that profilin inhibits the hydrolysis of PIP2 by unphosphorylated phospholipase C (PLC) but not the hydrolysis by PLC phosphorylated by epidermal growth factor receptor tyrosine kinase (14, 15). These results raise the interesting possibility that actin-modulating proteins function as a negative regulator of the phosphoinositide signaling pathway in quiescent cells (14, 15). In this report, we show that the dodecapeptide as well as cofolin binds to PIP2 molecules and inhibits PIP2 hydrolysis by PLC.

MATERIALS AND METHODS

Proteins—Recombinant cofolin was expressed in Escherichia coli and then purified to homogeneity as described previously (16). PLC-γ, and PLC-δ were purified from bovine thymus and PLC-γ, was from bovine spleen as described previously (17). PLC-δ, was purified from bovine brain as described previously (23).

Synthetic Peptides—A dodecapeptide, a pentapeptide, and a peptide corresponding to residues 150–160 of cofolin were synthesized as described previously (6).

Chemicals—Phosphatidylinositol (PI), phosphatidylinositol 4-monophosphate (PIP), phosphatidylinositol 4,5-bisphosphate (PIP2), phosphatidylserine (PS), phosphatidylcholine (PC), inositol 1,4,5-trisphosphate (IP3), and 1-oleoyl-2-acetyl-glycerol were all purchased from Sigma. The lipids were dissolved in water to a final concentration of 1 mg/ml and sonicated three times for 10 s in a sonicator. IP3 was dissolved in water to a final concentration of 0.1 mg/ml. The suspensions were frozen at −80 °C in 0.1-ml aliquots. The lipid solution was quickly thawed in warm water and sonicated in a sonicator three times for 10 s just before use.
Assay for Actin Polymerization Using Pyrene-labeled Actin—In the presence or absence of the synthetic peptide and either lipids or IP₃, polymerization of G-actin (5 μM, 3.6% pyrene actin) was monitored at 25 °C by the changes in fluorescence intensity of pyrene labeled to actin in a buffer containing 90 mM KCl, 20 μM ATP, 10 μM CaCl₂, and 4 mM Pipes, pH 7.0. The pyrene-labeled actin was prepared as described (1, 18). The fluorescence intensity was measured with a Hitachi 650–10S fluorescence spectrophotometer, and is shown as arbitrary units. The excitation and emission wavelengths were 365 and 407 nm, respectively.

**Phosphoinositide Binding Sequence of Cofilin**

The synthetic dodecapeptide, WAPECAPLKSKM, corresponding to Trp¹⁰⁴-Met¹¹⁵ of cofilin inhibited actin polymerization as previously reported (Fig. 1, and Ref. 6). We have found that the inhibition of actin polymerization by the dodecapeptide is canceled by PIP₃ in a dose-dependent manner as shown in Fig. 1a. About an equimolar PIP₂ to the dodecapeptide was required for the complete cancellation (Fig. 1a).

Fig. 1b shows the effects of various lipids and IP₃ on the inhibitory effect of the dodecapeptide on actin polymerization. In addition to PIP₂, PIP (●) and PI (■) canceled the inhibition of actin polymerization by the dodecapeptide, while PC (○), PS (△), 1-oleoyl-3-acetylglycerol (◇), or IP₃ (●) did not affect the activity of the dodecapeptide at all. Thus, the inhibitory effect of the dodecapeptide on actin polymerization was inhibited specifically by phosphoinositides. Because phosphoinositides do not affect the actin polymerization and do not bind to actin (11), these results suggest that the phosphoinositides block the interaction of the dodecapeptide with actin by binding to the dodecapeptide.

To show the binding of the dodecapeptide to the phosphoinositides directly, we carried out the gel filtration chromatography. In gel filtration on the Sephadex G-25 column, PIP₂ or PIP eluted at void volume while the dodecapeptide alone eluted at the position corresponding to an apparent molecular weight of ~1000 (Fig. 2, ○). When the dodecapeptide mixed with PIP₂ or PIP was passed through the column, however, the dodecapeptide co-eluted with PIP₂ or PIP at void volume (Fig. 2, ●). This indicated that the dodecapeptide binds to PIP₂ or PIP. In contrast, neither PC nor PS affected the elution position of the dodecapeptide (Fig. 2, ▲). A synthetic peptide corresponding to residues 150–166 of cofilin, that does not inhibit actin polymerization at all (6), eluted by itself at the position corresponding to an apparent molecular weight of ~2000. Neither PIP₂ nor PIP affected the elution position of this 17-amino acid synthetic peptide (data not shown).

These results suggest that phosphoinositides such as PIP₂ and PIP specifically bind to the dodecapeptide, that is, the actin-binding sequence of cofilin. Thus, the inhibition of interaction of cofilin with actin by phosphoinositides may be accounted for by assuming that phosphoinositides and actin compete for binding to the same site, the dodecapeptide sequence, in cofilin.

As the dodecapeptide as well as cofilin was found to bind to PIP₂ tightly, we tested the effect of cofilin and the dodecapeptide on PIP₂ hydrolysis by PLC. Cofilin inhibited the hydrolysis of PIP₂ by PLC-γ₂ in a dose-dependent manner (Fig. 3a). The rate of hydrolysis was 0 at molar ratios of 1 cofilin per 2.5–3 PIP₂ molecules. The stoichiometry of cofilin

![Fig. 1. Phosphoinositide sensitivity of the synthetic dodecapeptide.](image)

![Fig. 2. PIP₂ specifically binds to the dodecapeptide.](image)
Phosphoinositide Binding Sequence of Cofilin

The concentrations of the synthetic peptides required for the half-maximal inhibition of the initial rate of actin polymerization (assay conditions were the same as in Fig. 1 except for 3 µm actin containing 6% pyrene actin) and for the half-maximal inhibition of PIP₂ hydrolysis by PLC-γ₂ (as in Fig. 3) were determined. As both abilities of the synthetic pentapeptide, LKSKM, were weak, the data for the pentapeptide cannot be accurately determined and are rough estimates.

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<th>WAPECAPLKSKM</th>
<th>LKSKM</th>
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<td>Half-maximal inhibition</td>
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<td>1200</td>
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<td>of actin polymerization</td>
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<td>of PIP₂ hydrolysis by PLC</td>
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TABLE II

Comparison of activities of the dodecapeptide and the pentapeptide

Both cofilin and the dodecapeptide inhibit the hydrolysis of PIP₂ by PLC-γ₂ in a dose-dependent manner (Fig. 3b). At a molar ratio of 10 dodecapeptides per 1 PIP₂ molecule, the rate of hydrolysis was almost 0. Neither actin nor the other synthetic peptide, corresponding to residues 150–166 of cofilin, affected the rate of PIP₂ hydrolysis by PLC-γ₂ (data not shown). Both cofilin and the dodecapeptide also inhibited PIP₂ hydrolysis by other isoforms of PLC, PLC-β₁, PLC-γ₁, and PLC-δ (Table I). The inhibitory effect of cofilin and the dodecapeptide on PIP₂ hydrolysis did not markedly depend on the PLC isoforms used, although the inhibitory effect on PLC-β₁ might be the smallest. The dodecapeptide and cofilin may compete with PLC for interacting with PIP₂ and therefore inhibit PIP₂ hydrolysis by PLC. These results suggest the possibility that not only profilin but also cofilin may bind to PIP₂ on the membrane and maintain the very low rate of PIP₂ hydrolysis in resting cells.

Because the basic residues (Lys₁¹¹² and Lys₁¹¹⁴) in the dodecapeptide sequence are thought to be involved in the interactions of cofilin with the acidic residues of the N-terminal segment of actin and the acidic polar head of phosphoinositides, we assume that the latter portion of the dodecapeptide, LKSKM, might be important for cofilin binding to actin and phosphoinositides. Then, we examined whether the synthetic pentapeptide, LKSKM (residues 111–115 of cofilin), interacts with actin and phosphoinositides, and found that the pentapeptide has abilities to inhibit actin polymerization in a phosphoinositide-sensitive manner and to inhibit PIP₂ hydrolysis by PLC-γ₂, although both abilities are weak. The concentrations of the pentapeptide required for 50% decrease in the initial rate of actin polymerization and 50% inhibition of PIP₂ hydrolysis by PLC-γ₂ were roughly 50 and 20 times higher than those of the dodecapeptide, respectively (Table II). These results suggest that although this pentapeptide segment containing 2 lysine residues contributes to the actin and PIP₂ binding of cofilin, the N-terminal portion (WAPECAP) of the dodecapeptide is also necessary for full activities of the dodecapeptide.

In summary, the dodecapeptide sequence (Trp¹⁰⁴-Met¹¹⁵) of cofilin has the ability to interact with both actin and phosphoinositides, and thus may be responsible for cofilin’s abilities to interact with actin and to inhibit PIP₂ hydrolysis by PLC by binding to PIP₂. Thus, the dodecapeptide sequence may constitute a multifunctional region of cofilin. As the sequence very similar to the dodecapeptide exists in a family of actin-depolymerizing proteins, destrin (19, 20), depactin (21), and actin depolymerizing factor (22) as reported previously (6), this sequence region may constitute a domain responsible for not only actin depolymerizing activity but also PIP₂ binding activity of these proteins. These actin-depolymerizing proteins in concert with profilin may function as negative regulators of the phosphoinositide signaling pathway in addition to functioning as regulators of actin cytoskeleton.

REFERENCES

Phosphoinositide Binding Sequence of Cofilin