Inhibition of the Interactions of Cofilin, Destrin, and Deoxyribonuclease I with Actin by Phosphoinositides*

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Cofilin is a widely distributed actin-modulating protein that has the ability to bind along the side of F-actin and to depolymerize F-actin in a pH-dependent manner. We found that phosphatidylinositol (PI), phosphatidylinositol 4-monophosphate (PIP), and phosphatidylinositol 4,5-bisphosphate (PIP₂) inhibited both actions of cofilin in a dose-dependent manner, while inositol 1,4,5-triphosphate (IP₃), 1-oleoyl-2-acetylglycerol (OAG), phosphatidylserine (PS), or phosphatidylcholine (PC) had little or no effect on them. Gel filtration analyses showed that PIP₂ bound to cofilin and thereby inhibited the binding of cofilin to G-actin. Destrin is a mammalian, pH-independent actin-depolymerizing protein. The actin-depolymerizing activity of destrin was also inhibited by PI, PIP, and PIP₂, but not by IP₃, OAG, PS, or PC. In addition, we found further that an actin-depolymerizing activity of bovine pancreas deoxyribonuclease I, a G-actin-sequestering protein, was inhibited by PIP and PIP₂, but not by PI, IP₃, OAG, PS, or PC. These results together with previous findings (Lassening, T., and Lindberg, U. (1985) Nature 314, 472-474; Janmey, P. A., and Stossel, T. P. (1987) Nature 325, 362-364) suggest that the sensitivity to polyphosphoinositides may be a common feature in vitro among actin-binding proteins that can bind to G-actin and regulate the state of actin polymerization.

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The abbreviations used are: SDS, sodium dodecyl sulfate; PIP, phosphatidylinositol 4,5-bisphosphate; PIP₂, phosphatidylinositol 4,5-bisphosphate; PI, phosphatidylinositol 4-monophosphate; PI, phosphatidylinositol; IP₃, inositol 1,4,5-triphosphate; OAG, 1-oleoyl-2-acetylglycerol; PS, phosphatidylserine; PC, phosphatidylcholine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Pipes, piperazine-N,N'-bis(2-ethanesulfonic acid); DTT, dithiothreitol.

MATERIALS AND METHODS

Proteins—Actin was prepared from rabbit skeletal muscle by the method of Spudich and Watt (21) and further purified by Sephadex G-100 gel filtration column equilibrated with 2 mM Hepes, 0.1 mM CaCl₂, 0.01% NaN₃, 0.2 mM ATP, and 0.05 mM DTT, pH 7.9. Porcine brain cofilin was purified by the previously described method (22). The method of preparing profilin expressed in Escherichia coli was previously described (15). Bovine brain cofilin cDNA containing the entire open reading frame was ligated to the downstream of lacZ in the expression vector pUC19. Cofilin was then expressed in E. coli HB101, and the extract of HB101 was prepared by lysozyme treatment followed by freeze-thawing. Cofilin was then purified by hydroxyapatite chromatography and gel filtration. We call this cofilin recombinant cofilin in this paper. Recombinant cofilin has an additional sequence, Thr-Met-He-Thr-Pro-Ser-Ser-Gly-Asn on the authentic N terminus of porcine brain cofilin. Therefore, its apparent sequence homologous to that of gelsolin, villin, fragmin, severin, and Acanthamoeba profilin in its C-terminal portion (7).

Destrin is a mammalian actin depolymerizing protein with an apparent molecular mass of 19 kDa on SDS-polyacrylamide gel electrophoresis (8-10). Similar low molecular mass (15–19 kDa) actin-binding proteins have been identified in other organisms: actin-depolymerizing factor in chick embryo brain (11,12), depactin in echinodermatous oocytes and eggs (13), and actophorin in Acanthamoeba (14). These proteins can rapidly depolymerize F-actin in a pH-independent manner by taking actin molecules away from the entire length of F-actin. Recent CDN cloning studies have shown that the amino acid sequence of destrin, consisting of 165 amino acid residues, is very similar (71% identical) to that of cofilin which consists of 168 amino acid residues (7, 15).

It has been reported that polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate (PIP₂) and phosphatidylinositol 4-monophosphate (PIP) cause the dissociation of profilactin by interacting with profilin in vitro (16). The function of Ca²⁺-sensitive actin-severing proteins, gelsolin and villin, is also modulated by PIP and PIP₂ in vitro. These polyphosphoinositides can inhibit their actin-severing activities and induce dissociation of gelsolin-actin complex (17-19). Recently, Hartwig et al. (20) have suggested the possibility of the interaction of gelsolin with polyphosphoinositides in vivo, on the basis of their observation that part of gelsolin localizes with plasma and intracellular membranes.

It is interesting to examine whether phosphoinositides affect the interaction of cofilin or destrin with actin, because cofilin and destrin belong to another class of actin-binding proteins with gelsolin, villin, and profilin. In this paper, we show that phosphoinositides inhibit the binding of cofilin to F-actin, the actin-depolymerizing activity of cofilin and destrin, and the actin-depolymerizing activity of DNase I, a G-actin-sequestering protein. We further demonstrate the direct binding of PIP₂ to cofilin.
molecular mass on SDS-polyacrylamide gel electrophoresis is a little larger than that of porcine brain cofilin. The mode of interaction of recombinant cofilin with actin was indistinguishable from that of porcine brain cofilin (Ref. 15, see "Results"). Destrin was purified from porcine brains by using DNase I affinity column chromatography as previously described (9, 10). Bovine pancreas DNase I was purchased from Sigma. DNase I gave no other contaminating bands on SDS-polyacrylamide gel electrophoresis.

Chemicals—Phosphatidylinositol (PI), PIP, PIP2, phosphatidylserine (PS), phosphatidylincholine (PC), inositol 1,4,5-triphosphate (IP3), and 1-oleoyl-2-acetylglycerol (OAG) were all purchased from Sigma. The lipids except IP3 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.

Assays for the Binding of Cofilin to F-actin and the Actin-depolymerizing Activities of Cofilin, Destrin, and DNase I—Recombinant cofilin, porcine brain cofilin, destrin, or DNase I was first incubated with lipids or IP3 for 10 min at 25 °C, then the aliquot of polymerized actin was added, and the solution (final 100 ml) was incubated at 25 °C for various times. The reaction mixture was centrifuged at 356,000 × g for 15 min at 25 °C. The supernatant was saved and the pellet was suspended in 100 ml of 1% SDS. 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 mg/lane, in our SDS-polyacrylamide gel electrophoretic experiments, we adjusted the sample volume of pellet or supernatant fractions electrophoresed in one lane within this range. After staining and destaining the gels, the staining intensities of both the pellet and supernatant fractions were determined with a densitometer (Beckman), and the amount of each protein in pellets and supernatants was determined by using the equation: (Amount of each protein in pellet or supernatant) = [(staining intensity of pellet or supernatant) × (amount of each protein in the reaction solution)]/ (staining intensity of pellet or supernatant) × (amount of protein in the reaction solution). SDS-polyacrylamide gel electrophoresis was performed on 10% polyacrylamide gels as described by Lasemml (23).

Other Assays—DNase I inhibition assay was performed as described previously (1, 25). Changes in fluorescence intensity of pyrene-labeled actin were measured at 25 or 37 °C with a Hitachi 650-10S fluorescence spectrophotometer. Excitation and emission wavelengths were 365 and 407 nm, respectively.

RESULTS

Effect of Polyphosphoinositides on the Binding of Cofilin to F-actin—Recombinant cofilin was purified to homogeneity as described under "Materials and Methods" and was indistinguishable from porcine brain cofilin in its interaction with actin (Ref. 15, see below). Porcine brain cofilin and recombinant cofilin bound to F-actin in the same dose-dependent manner at pH 7.0, and the saturation levels of both cofilins were about 0.7-0.8:1 molar ratio of bound cofilin to actin molecules in F-actin under the assay conditions of Fig. 1. When increasing concentrations of recombinant cofilin were reacted with F-actin in the presence of a fixed concentration of brain cofilin, the molar ratio of recombinant cofilin to brain cofilin in the F-actin pellet was nearly the same as that in the initial reaction mixture (Table I). These results suggest that both cofilins have nearly the same affinity for F-actin.

As shown in Fig. 1A, the amount of porcine brain cofilin bound to F-actin was decreased by PIP2 in a dose-dependent manner. In the presence of a 50:1 molar ratio of PIP2 to cofilin, cofilin could not bind to F-actin at all. The binding of recombinant cofilin to F-actin was also inhibited by PIP2 in almost the same dose-dependent manner (Fig. 1B). We further investigated the effects of other phospholipids and IP3 on the binding of recombinant cofilin to F-actin. PIP2 had almost the same inhibitory effect as PIP3, and PI had a slightly weaker inhibitory effect than PIP and PIP3. On the other hand, PC, PS, OAG, and IP3 did not inhibit the cofilin binding to F-actin at all (Fig. 1D). It should be noted that none of the phospholipids and IP3 affected the polymerization level of actin in the absence of cofilin.

It is known that an increase in the fluorescence intensity of pyrene labeled to actin accompanies polymerization of actin (25). When F-actin (final concentration 4.0 μM) containing 1% pyrene-labeled actin was mixed with recombinant cofilin (final concentration 4.1 μM) at pH 8.3, the fluorescence intensity of the pyrene was decreased to nearly zero because of the binding of cofilin to actin, as previously reported (1). Prior incubation of cofilin with PIP2 (final concentration 360 μM) inhibited this cofilin-induced decrease of fluorescence intensity almost completely (data not shown). This result also suggests that the binding of cofilin to F-actin is inhibited by PIP2. When PIP2 (final concentration 360 μM) was added to the mixture of F-actin (final concentration 4.0 μM) and cofilin (final concentration 4.1 μM) at 37 °C, the fluorescence inten-
sity was gradually increased from nearly the zero level to that of F-actin in the absence of cofilin within 30 min (data not shown). This result indicates that dissociation of cofilin from F-actin occurs by the action of PIP₂.

**Effect of Polyphosphoinositides on the Actin-depolymerizing Activity of Cofilin**—Porcine brain cofilin has a pH-dependent actin-depolymerizing activity (6). Recombinant cofilin also depolymerized F-actin in a pH-dependent manner (15). The actin-depolymerizing activity at pH 8.3 and the relatively weak actin-depolymerizing activity at pH 7.0 of both brain cofilin (Fig. 2A) and recombinant cofilin (Fig. 2, B and C) were reduced to the half level in the presence of about a 20:1 molar ratio of PIP₂ to cofilin and were almost completely inhibited in the presence of 50–100 molar ratio of PIP₂ to cofilin. PIP and PI also inhibited the actin-depolymerizing activity of cofilin (Fig. 2, B and C) in a concentration-dependent manner. But, PC, PS, OAG, and IP₃ had little or no inhibitory effect on it (Fig. 2, B and C).

It has been shown that incubation of PIP₂ with divalent cations prior to mixing with gelsolin decreases the ability of PIP₂ to inhibit the actin severing function of gelsolin (18). We have then examined the effect of Mg²⁺ ions on the action of PIP₂, PIP, and PI on cofilin. Prior to the mixing with recombinant cofilin, PIP₂, PIP, or PI was incubated in the solution containing 2 mM MgCl₂ at 25 °C for 20 min. In this case, the inhibitory effects of PIP₂, PIP, or PI on the cofilin binding to F-actin and on the actin-depolymerizing activity of cofilin were decreased by 80%. On the other hand, addition of Mg²⁺ after mixing PIP₂ with cofilin caused no decrease in the inhibitory effects of PIP₂ (data not shown). These results are consistent with the previous report examining the effect of Mg²⁺ ions on the inhibitory effect of PIP₂ on the severing function of gelsolin (18).

**Interaction of PIP₂ with Cofilin**—We have examined whether PIP₂ binds to cofilin or actin by gel filtration chromatography. Sephadex G-100 gel filtration column chromatographies were carried out in the presence of 100 mM NaCl. PIP₂ forms small micelles of molecular mass 0.3,000 Da (26) and was eluted near the void volume (Fig. 3A, —). When a low concentration of G-actin with (C) or without (●) PIP₂ was passed through the column, actin was eluted at the position corresponding to a monomer in both cases (Fig. 3A), indicating that PIP₂ does not bind to actin under the conditions used. In contrast, the elution position of cofilin changed when cofilin mixed with PIP₂ was passed through the column. Almost all the cofilin was co-eluted with PIP₂ near the void volume (Fig. 3B, ▲). This clearly indicates that PIP₂ binds to cofilin.

Next, a mixture of cofilin and actin (2:1 molar ratio) without (Fig. 3C) or with (Fig. 3D) PIP₂ was passed through the column. In Fig. 3C, elution profiles of actin (●) and cofilin (▲) are shown. Without PIP₂ about 70% of the actin was eluted at the position of the actin-cofilin complex and the remainder was eluted at the position of G-actin, while about...
25% of the coflin was eluted at the position of actin-cofilin complex. Thus, the molar ratio of coflin to actin at the position of the actin-cofilin complex was about 0.6. This relatively low ratio may result from dissociation of coflin from actin during the elution because of the low concentration of actin and coflin. When a mixture of coflin and actin withPIP <sub>2</sub> (50:1 molar ratio to coflin) was passed through the column, coflin was co-eluted with PIP <sub>2</sub> near the void volume (Fig. 3D, Δ) and almost all actin eluted at the position corresponding to a monomer (Fig. 3D, ○). These results clearly indicate that PIP <sub>2</sub> binds to coflin and thereby inhibits the interaction of coflin with actin.

**Effect of Phosphoinositides on the Actin-depolymerizing Activity of Destrin**—Destrin is a mammalian, pH- and Ca<sup>2+</sup>-independent actin-depolymerizing protein (8, 9), whose amino acid sequence has been revealed to be very similar to that of coflin (7, 15). Therefore, we have asked whether the activity of destrin is also regulated by phosphoinositides. Fig. 4 shows that PIP <sub>2</sub> (●) and PIP (○) inhibited the ability of destrin to depolymerize F-actin in nearly the same dose-dependent manner. The half-maximal inhibitory effect was seen at about a 10:1 molar ratio of PIP or PIP <sub>2</sub> to destrin. Therefore, their inhibitory effect on the activity of destrin is slightly stronger than that of coflin's activity. PI showed a slightly weaker inhibitory effect on the activity of destrin than PIP and PIP <sub>2</sub>, but PS, PC, IP <sub>3</sub>, and OAG had little or no inhibitory effects (Fig. 4).

**Effect of Polyphosphoinositides on the Actin-depolymerizing Activity of DNase I**—We found that the nuclease activity of DNase I was inhibited by PIP <sub>3</sub>. The inhibition by PIP <sub>3</sub> was dose-dependent and about an 80:1 molar ratio of PIP <sub>2</sub> to DNase I was required for the half-maximal inhibition (data not shown). It has been shown that DNase I can induce a relatively slow depolymerization of F-actin by sequestering monomeric actins which are in equilibrium with F-actin (27–29). Then, we have examined the effect of phosphoinositides on the actin-depolymerizing activity of DNase I. PIP <sub>2</sub> and PIP <sub>3</sub> inhibited the actin-depolymerizing activity of DNase I in a dose-dependent manner and the half-maximal inhibitions were obtained at about 25:1 and 50:1 molar ratios of PIP <sub>2</sub> and PIP to DNase I, respectively (Fig. 5). These inhibitory effects of PIP <sub>2</sub> and PIP on DNase I were slightly weaker than those on coflin and destrin. Although PI inhibited the actions of coflin and destrin (Figs. 1, 2, and 4), it did not inhibit the actin-depolymerizing activity of DNase I (Fig. 5, ○). PS, PC, IP <sub>3</sub>, and OAG had little or no effect on the action of DNase I (Fig. 5) as in the case of coflin or destrin. Thus, the sensitivity of DNase I to phosphoinositides is somewhat different from that of coflin or destrin.

**DISCUSSION**

It has already been reported that polyphosphoinositides affect the functions of a G-actin-sequestering protein, profilin (16), and Ca<sup>2+</sup>-sensitive actin-severing/capping proteins, gelsolin (17–19) and villin (19) in *in vitro*. In this study, we have found that besides these actin-regulatory proteins, an F-actin-side-binding and -depolymerizing protein, coflin, and actin-depolymerizing/severing protein, destrin, and a G-actin-sequestering protein, DNase I, are sensitive to polyphosphoinositides.

Gel filtration column chromatography on Sephadex G-100 revealed that PIP <sub>3</sub> binds to coflin but not to actin. It has also been reported that PIP <sub>3</sub> binds to profilin (16) and gelsolin (18) in *in vitro*. As PIP <sub>3</sub> inhibits the functions of destrin, DNase I and villin, PIP <sub>3</sub> may bind to these proteins, too. Thus, at least these six kinds of actin-binding proteins have the ability to bind to PIP <sub>3</sub>. It is interesting that the cross-linking site in the actin sequence for DNase I, the segment of 48–52 amino acid residues (31), is different from the sites for gelsolin and coflin, which have been determined to be the N-terminal and/or C-terminal portions of actin (3, 30). Furthermore,
these actin-binding proteins are clearly different from one another in their mode of interaction with actin as described above. Therefore, despite the common feature of the sensitivity to PIP2, the molecular mechanism underlying the interaction with actin may not be common among these proteins.

Previous studies using gelsolin fragments generated by limited proteolysis (32) or by gene truncation of plasma gelsolin cDNA (33) revealed that the PIP2 binding site on the gelsolin sequence is on an 11-amino acid sequence (150-160 amino acid residues). It is also suggested that this 11-amino acid sequence may be the F-actin-side-binding site (33). Neither cofilin nor destrin has a sequence similar to the 11-amino acid sequence. Therefore, we do not know at present whether a consensus motif for the phosphoinositide-binding sites of these actin-binding proteins exists.

Both PIP2 and PIP inhibit the function of gelsolin, villin, protein, cofilin, destrin, and DNase I in vitro. PI has an inhibitory effect on cofilin (Figs. 1 and 2) and destrin (Fig. 4) but has little or no effect on gelsolin (18), profilin (16), and DNase I (Fig. 5). The effect of PI on the function of villin has not yet been reported. Thus, there is a slight but clear difference in the sensitivity to different phosphoinositides between these actin-binding proteins. Cofilin and destrin have distinct but similar actin-modulating functions in vitro and their amino acid sequences are highly homologous (15). This study has shown that, in addition to these similarities, cofilin and destrin share the common feature of the sensitivity to PIP2, PIP, and PI.

It has not been determined whether the function of other actin-binding proteins, such as α-actinin, filamin, and tropomyosin, is inhibited by phosphoinositides, although it was reported that α-actinin forms a complex with diacylglycerol and palmitic acid (34). It can be concluded, however, that a sensitivity to polyphosphoinositides in vitro is a common feature at least among the actin-binding proteins that bind to G-actin and regulate the polymerization state of actin.

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