Profilin and Gelsolin Stimulate Phosphatidylinositol 3-Kinase Activity†

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ABSTRACT: Actin-binding proteins such as profilin and gelsolin bind to phosphatidylinositol (PI) 4,5-bisphosphate (PI 4,5-P_2) and regulate the concentration of monomeric actin. We report here that profilin and gelsolin stimulate PI 3-kinase-mediated phosphorylation of PI 4,5-P_2 (lipid kinase activity) in a concentration-dependent manner. This effect is specific to profilin and gelsolin because other cytoskeletal proteins such as tau or actin do not affect PI 3-kinase activity. In addition to lipid kinase activity, PI 3-kinase also has protein kinase activity: it phosphorylates proteins (p85 subunit of PI 3-kinase). However, the protein kinase activity of PI 3-kinase was not affected in the presence of profilin. Kinetic analysis, as a function of varying concentrations of ATP and PI 4,5-P_2, showed that profilin affects the V_{max} of PI 3-kinase without affecting km. Profilin may also affect PI 3-kinase activity by its direct association to the enzyme because dot-blot analysis using antibody to glutathione *S*-transferase (GST) suggested that GST−85 kDa, a fusion protein of PI 3-kinase, binds to profilin. However, PI 3-kinase did not affect the actin-sequestering ability of profilin (determined by pyrene-labeled actin), which indicates that actin and p85 do not share a common binding site on profilin. These studies suggest that profilin and gelsolin may control the generation of 3-OH phosphorylated phosphoinositides, which in turn may regulate the actin polymerization.

Several growth factors such as epidermal growth factor (EGF), nerve growth factor (NGF), platelet derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), phorbol esters, and ras cause membrane ruffling in different cell types (Brunk et al., 1976; Chinkers et al., 1979; Connolly et al., 1979; Goshima et al., 1984; Bar-Sagi & Feramisco, 1986; Kadowaki et al., 1986; Mellstrom et al., 1988). The membrane ruffling occurs as a result of alterations in cell morphology and cytoskeletal architecture, which follows the binding of growth factors to receptors. The molecular events responsible for ruffle formation are not known, but it is believed that actin polymerization at the inner surface of the plasma membrane plays a crucial role (Mellstrom et al., 1988). Profilin and gelsolin are two major actin-sequestering proteins that are considered to regulate the assembly of actin filaments. Addition of profilin to F-actin always results in net inhibition of filament assembly. However, under certain conditions, profilin can promote actin polymerization by increasing the exchange of nucleotide bound to actin (Goldschmidt-Clermont et al., 1992) and by lowering the critical concentration of actin at the fast growing "barbed" end (Pantaloni & Carlier, 1993). Profilin and gelsolin also bind with high affinity to phosphatidylinositol 4,5-bisphosphate (PI 4,5-P_2),[1] a membrane component (Janmey, 1995), and inhibit phospholipase C (PLC)-mediated hydrolysis of PI 4,5-P_2 (Goldschmidt-Clermont et al., 1990; Banno et al., 1992). However, when PLC is phosphorylated at the specific tyrosine residues during agonist-coupled activation of the cell, it can overcome the protective effect of profilin and it can hydrolyze PI 4,5-P_2 (Goldschmidt-Clermont et al., 1991). Several investigators have suggested that dissociation of profilin from the actin/profilin complex by PI 4,5-P_2 may be the key event for the initiation of actin polymerization (Lassing & Lindberg, 1985; Janmey, 1995). Research in actin polymerization gained momentum after the discovery of 3-OH phosphorylated phosphoinositides. These lipids are produced by the action of PI 3-kinase on PI, PI 4-P, and PI 4,5-P_2 (Auger et al., 1989; Hawkins et al., 1992). Since 3-OH phosphorylated phosphoinositides (novel phosphoinositides) are not substrates of PLC (Lips et al., 1989), many investigators proposed that novel lipids may be true regulators of actin polymerization (Eberle et al., 1990; Arcaro & Wymann, 1993; Wymann & Arcaro, 1994). Increase in levels of PI 3,4,5-P_3 during actin polymerization in fact support this view (Eberle et al., 1990).

PI 3-kinase has an apparent size of 190 kDa and is a heterodimer consisting of 85-kDa and 110-kDa subunits (Carpenter et al., 1990; Shibasaki et al., 1991; Morgan et al., 1990). The 85 kDa subunit is phosphorylated on serine, threonine, and tyrosine residues after middle-T antigen transformation or exposure of fibroblasts to PDGF (Kaplan et al., 1987; Escobedo et al., 1991; Courtneidge & Heber, 1987). Recently, it has been reported that the 110-kDa subunit of PI 3-kinase contains two catalytic activities: (i) Mg^{2+}-dependent lipid kinase activity, which phosphorylates PI, PI 4-P, and PI 4,5-P_2, and (ii) Mn^{2+}-dependent protein kinase activity, which phosphorylates the 85-kDa subunit of PI 3-kinase (Dhand et al., 1994; Chauhan et al., 1995), and IRS-1 (Lam et al., 1994). The 85-kDa subunit of PI 3-kinase contains one SH3 and two SH2 regions homologous to the nonkinase regions of pp60 c-src (Otsu et al., 1991) and appears to mediate the specificity of association of PI

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[1] Abbreviations: PI, phosphatidylinositol; PI 4-P, phosphatidylinositol 4-phosphate; PI 4,5-P_2, PI 4,5-bisphosphate; PI 3-P, phosphatidylinositol 3-phosphate; PI 3,4-P_2, phosphatidylinositol 3,4-bisphosphate; PI 3,4,5-P_3, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C; PLC, phospholipase C; PI 3-kinase, phosphatidylinositol 3-kinase.

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3-kinase with protein tyrosine kinases of both receptor and nonreceptor classes, whereas the 110-kDa subunit is considered to be the catalytic component of PI 3-kinase (Otsu et al., 1991; Dhand et al., 1994).

Since profilin and gelsolin bind to phosphoinositides, we investigated if these proteins regulate PI 3-kinase activity. We report here that profilin and gelsolin stimulate PI 3-kinase activity in a concentration-dependent manner. Other cytoskeleton proteins such as tau and actin do not affect its activity. We suggest that profilin and gelsolin may help in conversion of PI 4,5-P_2 into 3-OH phosphorylated phosphoinositides, which may be an important step in the regulation of actin polymerization.

EXPERIMENTAL PROCEDURES

Materials. DEAE-Sepharose (Fast Flow), S-Sepharose (Fast Flow), and Mono Q (5/5) were purchased from Pharmacia. [γ-^{32}P]ATP (7000 Ci/mmol) was from ICN, and Ecoscint A was from National Diagnostics. Gelsolin, PS, PI, PI 4-P, PI 4,5-P_2, and a mixture of phosphoinositides (containing 5−20% PI 4-P, 5−20% PI 4,5-P_2, and the remainder PS + PI) were obtained from Sigma. Silica gel 60 plates were from E. Merck. Frozen bovine spleens were from Pel-Freeze, and glutathione Sepharose was from Molecular Probes. High Five insect cells were purchased from Invitrogen, and Ex-Cell 400 medium was from JRH Laboratories. The baculovirus recombinant vectors of the p85α and p110α subunits of PI 3-kinase were generous gifts from Prof. Waterfield, Ludwig Cancer Institute, London, U.K. The pGEX2Tp85α expression vector was kindly provided by Dr. Yasuhisa Fukui, University of Tokyo, Japan. Anti-GST monoclonal antibodies and the human isoform 3L tau were gifts from Drs. L. Kotula and K. Iqbal of this Institute, respectively. The tau isoform 3L that has three tandem C-terminal repeats was expressed in *Escherichia coli* BL21 (DE3) cells. All other chemicals were from Sigma.

Purification of Profilin. Profilin was purified from bovine spleen by poly-L-proline affinity chromatography, as described by Lindberg et al. (1988).

Actin. Actin was purified from rabbit skeletal muscle acetone powder by repeated polymerization and depolymerization, using the method of McLean-Fletcher and Pollard (1980). Three cycles yielded pure actin preparation. Pyrene-labeled actin was prepared as described by Kouyama and Mihashi (1981).

Expression of PI 3-Kinase in High Five Insect Cells. High Five insect cells were grown to 90% confluency in Ex-Cell 400 medium and were infected with recombinant p110α and p85α baculovirus vectors (MOI of 10). After 3 days of infection, the cells were centrifuged at 2000 rpm for 10 min and washed with phosphate-buffered saline buffer (PBS), pH 7.5. Cells were homogenized in 20 mM Tris-HCl, pH 7.5, containing 2 mM EDTA, 10 mM EGTA, 0.1% mercaptoethanol, 10 mM benzamidine, 1 μg/mL leupeptin, and 2 mM phenylmethylsulfonyl fluoride (PMSF) by using a Dounce homogenizer. The homogenate was then centrifuged at 100000*g* for 1 h at 4 °C. PI 3-kinase was purified from the supernatant, as described previously, by using DEAE-Sepharose, S-Sepharose, and Mono Q column chromatography (Chauhan et al., 1995).

Protein was estimated by the method of Bradford (1976) using bovine serum albumin as the standard or by measuring absorbance assuming an extinction coefficient of 0.62 cm^2 mg^{-1} at 290 nm for actin (Gordon et al., 1976) and of 1.2 cm^2 mg^{-1} at 280 nm for profilin (Segura & Lindberg, 1984).

Preparation of Liposomes. Organic solvents from the lipids (either phosphoinositides alone or 1:1 molar ratio of PS/PI, PI 4-P, or PI 4,5-P_2) were evaporated under argon. Liposomes were prepared by suspending lipid film in 20 mM Tris-HCl, pH 7.5, containing 0.1% mercaptoethanol and 1 mM EDTA and incubating it at 37 °C for 10 min, followed by sonication under argon to clearing.

Assay of Lipid Kinase Activity of PI 3-Kinase. The lipid kinase activity of PI 3-kinase was measured by the method of Carpenter et al. (1990), as described previously (Chauhan et al., 1995). The different cytoskeletal proteins (profilin, gelsolin, actin, or tau) were preincubated with phosphoinositides for 30 min at room temperature prior to the reaction. The assay mixture (25 μL) contained 4 mM $MgCl_2$, 50 mM KCl, 100 μM [γ-^{32}P]ATP, 0.25 mM EDTA, 20 mM Tris-HCl (pH 7.5), and 200 μM liposomes (100 μM PS/100 μM phosphoinositide or 200 μM phosphoinositides) and indicated concentrations of cytoskeletal proteins. The reaction was started at 37 °C with PI 3-kinase and terminated after 10 min by the addition of 105 μL of 1 N HCl, followed by 160 μL of chloroform and methanol (1:1, V/V). Samples were vortexed vigorously and centrifuged to separate the aqueous and organic phases. The organic phase (30 μL) was then transferred into a scintillation vial, dried, and counted for radioactive phosphoinositides. In some experiments, aliquots of the organic phase of samples were also analyzed for phosphorylated lipid products by separating them on oxalated silica gel 60 plates, as described earlier (Singh et al., 1993). The error for estimation of lipid kinase activity of PI 3-kinase was less than 10%.

Aggregation of Liposomes. Ca^{2+} or Mg^{2+} (2 mM) or profilin (10 μM) was added to liposomes (10 μM PS/10 μM PI 4,5-P_2) in 1 mL of 20 mM Tris-HCl, pH 7.5, and liposomal aggregation was studied by monitoring the light scattering at different time intervals at emission and excitation wavelengths of 320 nm using LS 3B fluorescence spectrometer (Perkin Elmer).

Actin Polymerization. Actin polymerization was studied by the method of Hofmann et al. (1992). In brief, 10% pyrene-labeled G-actin (1 μM) was incubated alone, or with profilin (1.4 μM), or profilin (1.4 μM) and p85α (2 μM), or profilin (1.4 μM) and PI 3-kinase (2 μg) in G-buffer (2 mM imidazole hydrochloride, pH 7.0, containing 0.2 mM ATP, 0.2 mM $CaCl_2$, 1 mM mercaptoethanol, and 0.02% sodium azide), and then transferred to F-buffer (G-buffer containing 2 mM $MgCl_2$ and 100 mM KCl). After 30 min, fluorescence was measured using an excitation wavelength of 365 nm and an emission wavelength of 407 nm in a Perkin Elmer LS 50B luminescence spectrometer.

Dot−Blot Analysis. It was performed by using an immunoblot assay kit from Bio-Rad as per the manufacturer's instructions. Profilin (1 μg) and/or actin (2 μg) were spotted onto a nitrocellulose membrane strip, blocked with 3% gelatin in TTBS, i.e., 0.05% Tween 20 in 20 mM Tris-HCl, pH 7.5, containing 0.5 M NaCl (TBS). The blocked strips were incubated with 10 μg/mL solution of p85αGST fusion protein (expressed in *E. coli* and purified by glutathione affinity chromatography) for 2 h, followed by five washes with TTBS. The washed membrane was incubated with GST monoclonal antibodies (1:1000 dilution). After 4 h, the

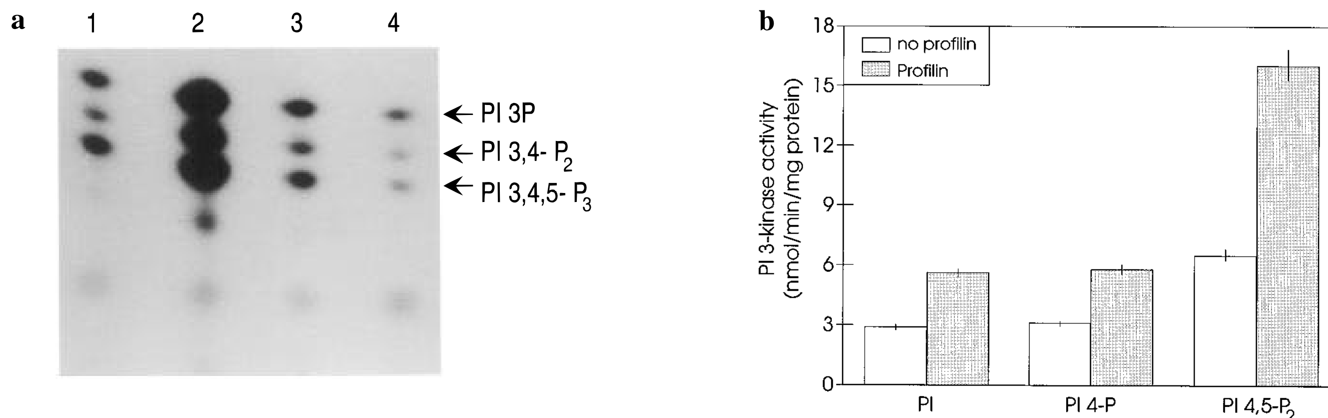


FIGURE 1: Stimulation of PI 3-kinase-mediated phosphorylation of a phosphoinositide mixture by profilin. PI 3-kinase was purified from High Five insect cells transfected with PI 3-kinase baculoviruses, as described in Experimental Procedures. The effect of profilin on PI 3-kinase-mediated phosphorylation of a phosphoinositide mixture was measured. The profilin (10 μM) and liposomes made up of 200 μM phosphoinositide mixture were preincubated for 30 min at room temperature. The assay mixture (25 μL) contained 20 mM Tris-HCl (pH 7.5), 4 mM EDTA, 50 mM KCl, 100 μM [γ-^{32}P]ATP, and liposomes or profilin/liposomes (panel a). The reaction was started at 37 °C with PI 3-kinase and terminated after 10 min by the addition of 105 μL of 1 N HCl. The reaction products were separated by TLC, followed by autoradiography as described in Experimental Procedures. The autoradiograph of phosphorylated products of phosphoinositides was as follows: lane 1, no profilin; lane 2, 10 μM profilin; lane 3, 10 μM profilin + 10 nM wortmannin; lane 4, 10 μM profilin + 0.02% NP-40. Stimulation of PI 3-kinase-mediated phosphorylation of 200 μM phosphoinositides (1:1 molar ratio of PS and PI, PI 4-P, or PI 4,5-P_2) by profilin was measured as described above. The radioactivity incorporated into the product was measured in the organic phase (30 μL), as described in Experimental Procedures. The results are expressed as Mean ± SE of four determinations.

membrane was washed four times with TTBS and then incubated with alkaline phosphatase-coupled secondary antibody. The unbound secondary antibody was washed with TBS, and the color developed by using the Bio-Rad immunoblot assay kit.

Protein Kinase Activity of PI 3-Kinase. Profilin was incubated with PI 3-kinase for 10 min at 37 °C, and the Mn^{2+}-dependent protein kinase activity of PI 3-kinase was measured as described earlier (Chauhan et al., 1995).

RESULTS

Effect of Profilin on PI 3-Kinase-Mediated Phosphorylation of Phosphoinositides. Figure 1 shows the effect of 10 μM profilin on PI 3-kinase-mediated phosphorylation of phosphoinositides. For our preliminary experiments, we used a mixture of phosphoinositides (i.e., PI, PI 4-P, and PI 4,5-P_2) obtained from Sigma. Profilin stimulated the phosphorylation of all three phosphoinositides by PI 3-kinase (Figure 1a). The stimulation of PI 3-kinase-mediated phosphorylation of phosphoinositides was inhibited by wortmannin (10 nM), a specific inhibitor of PI 3-kinase, and by NP-40 (0.02%), a detergent which is also known to inhibit PI 3-kinase (Figure 1a). The autoradiogram of the phosphorylated phosphoinositides showed that all three phosphoinositides were phosphorylated to a similar extent (Figure 1a). However, in this preparation of the phosphoinositides mixture (Sigma), the concentration of different phosphoinositides was not the same. Therefore, we compared the effect of profilin on the PI 3-kinase-mediated phosphorylation of PI, PI 4-P, or PI 4,5-P_2 at 100 μM concentrations (Figure 1b). The phosphorylation of phosphoinositides by PI 3-kinase was in the order of PI 4,5-P_2 > PI 4-P ≥ PI. This suggested that the profilin/PI 4,5-P_2 complex may be a preferred substrate for PI 3-kinase. Next, we studied the effect of different concentrations of profilin (0−14 μM) on PI 3-kinase activity. Profilin stimulated the PI 3-kinase-mediated phosphorylation of PI 4,5-P_2 in a concentration-dependent manner (Figures 2a and 2b), with maximum stimulation at 7.5 μM.

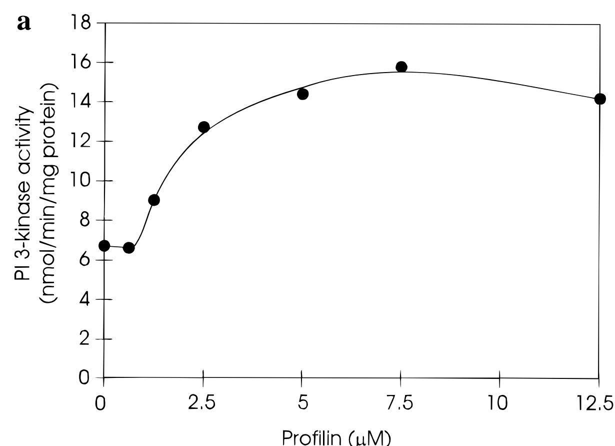
Effect of PI 4,5-P_2 and ATP Concentrations on Profilin-Mediated Stimulation of PI 3-Kinase Activity. PI 3-kinase activity was assayed in the presence or absence of 10 μM profilin as a function of PI 4,5-P_2 (20 to 160 μM) (Figure 3) and ATP (10 to 100 μM) (Figure 4). A Lineweaver-Burk plot of 1/PI 3,4,5-P_3 formed (PI 3-kinase activity) vs 1/[PI 4,5-P_2] or 1/[ATP] in the absence or presence of 10 μM profilin showed that profilin stimulated PI 3-kinase activity in a non-competitive manner with respect to ATP (increasing the V_{max} from 0.4 to 0.598 nmol of PIP_3 formed) and PI 4,5-P_2 (increasing the V_{max} from 0.102 to 0.238 nmol of PIP_3 formed) without affecting the K_m. Approximately 8% PI 4,5-P_2 was phosphorylated in the presence of optimum concentrations of profilin (7.5 μM) and PI 4,5-P_2 (80 μM).

Effect of Other Cytoskeletal Proteins on PI 3-Kinase Activity. In order to investigate if other cytoskeletal proteins also stimulate PI 3-kinase activity, we studied the effect of gelsolin, tau, and actin on PI 3-kinase activity. While gelsolin stimulated PI 3-kinase activity in a manner like profilin, no effect of actin and tau was observed (Figure 5).

Effect of Various Concentrations of Gelsolin on PI 3-Kinase-Mediated Phosphorylation of PI 4,5-P_2. PI 3-kinase activity was assayed in the presence of different concentrations of gelsolin (0−2 μM) by using PI 4,5-P_2 as a substrate. Similar to profilin, gelsolin also stimulated PI 3-kinase activity in a concentration-dependent manner (Figure 6).

Effect of Profilin on Protein Kinase Activity of PI 3-Kinase. As mentioned earlier, PI 3-kinase has intrinsic lipid kinase activity and protein kinase activity. In addition to phosphorylation of IRS-1, a 110-kDa subunit of PI 3-kinase can also phosphorylate its 85-kDa subunit. Therefore, we studied the effect of profilin on protein kinase activity, i.e., phosphorylation of the 85-kDa subunit. Profilin did not affect the phosphorylation of the 85-kDa subunit of PI 3-kinase (data not shown).

Effect of Profilin and Calcium on the Aggregation of PI 4,5-P_2 Liposomes. To investigate whether profilin induces



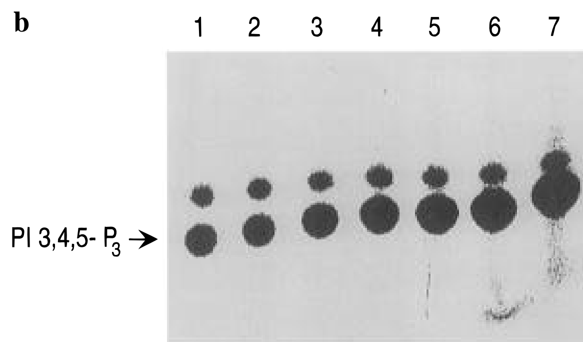


FIGURE 2: Effect of various concentrations of profilin on PI 3-kinase-mediated phosphorylation of PI 4,5-P_2. The indicated concentrations of profilin were incubated with 200 μM PS/PI 4,5-P_2 (1:1) for 30 min at room temperature, and the lipid kinase activity of PI 3-kinase was measured as described in Figure 1. An autoradiogram of the phosphorylated products of PI 4,5-P_2 in the presence of various concentrations of profilin was done by analyzing the lipid products on thin-layer chromatography (b), as described in Experimental Procedures. The different concentrations of profilin were lane 1, no profilin; lane 2, 0.67 μM; lane 3, 1.4 μM; lane 4, 2.7 μM; lane 5, 5.4 μM; lane 6, 8.4 μM; and lane 7, 14 μM profilin. The data are representative of three experiments done independently. The experimental error for all data points is less than 10%.

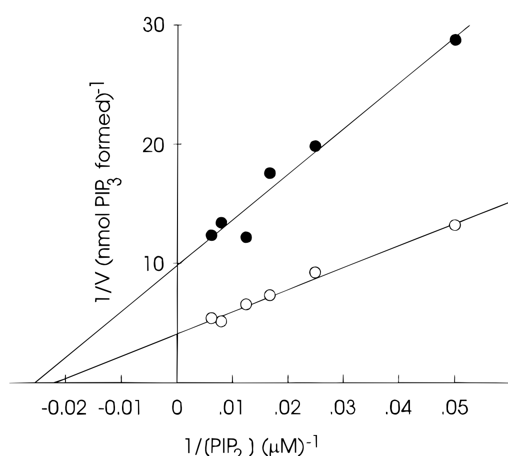


FIGURE 3: Effect of substrate (PI 4,5-P_2) concentration on profilin-mediated stimulation of PI 3-kinase activity. PI 3-kinase activity was measured as a function of PI 4,5-P_2 (20 to 200 μM) in the absence of profilin (●) and in the presence of 10 μM profilin (○). The data are representative of three experiments done independently. The experimental error for all data points is less than 10%.

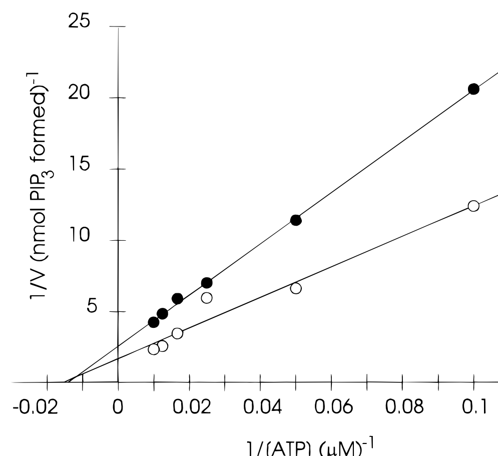


FIGURE 4: Profilin-mediated stimulation of PI 3-kinase activity as a function of ATP concentration. PI 3-kinase-mediated phosphorylation of PI 4,5-P_2 was measured at varying concentrations of ATP in the absence (●) and presence of 10 μM profilin (○). The data are representative of three experiments done independently. The experimental error for all data points is less than 10%.

stimulation of PI 3-kinase activity by affecting aggregation of PI 4,5-P_2 vesicles, we studied the effect of profilin (10 μM) and calcium or Mg^{2+} (2 mM) on aggregation of PI 4,5-P_2 vesicles. While calcium or magnesium increased the aggregation of PI 4,5-P_2 vesicles, profilin did not aggregate the liposomes (data not shown). These results are in agreement with the results of Janmey and Stossel (1989) who also did not observe aggregation of PI 4,5-P_2 vesicles in the presence of gelsolin or profilin.

Effect of p85 Subunit of PI 3-Kinase on Actin Polymerization. Because p85 subunit of PI 3-kinase binds to profilin, the effect of p85 on the actin-sequestering ability of profilin was measured by using pyrene-labeled G-actin as described in Experimental Procedures. The fluorescence spectra of polymerized actin showed that neither p85 nor intact PI 3-kinase (after 30 min of incubation) affects the binding of profilin to actin (data not shown). These results suggest that actin and p85 binding to profilin do not share a common binding site.

Binding of Profilin to the 85-kDa Subunit of PI 3-Kinase. We used GST antibody for studies on binding of profilin to the 85-kDa subunit of PI 3-kinase. Dot-blot analysis of GST-85-kDa binding to profilin using GST antibody showed that profilin and profilin/actin bind to the 85-kDa subunit, while no binding was observed with actin alone (Figure 7).

DISCUSSION

Phosphoinositides regulate the function of several intracellular proteins such as profilin (Lassing & Lindberg, 1985), gelsolin (Janmey & Stossel, 1987), α-actinin (Fukami et al., 1992), phospholipase D (Pertile et al., 1995), and protein kinase C (PKC) (Chauhan & Brockerhoff, 1988; Lee & Bell, 1991; Kochs et al., 1993; Singh et al., 1993). The SH2 (Rameh et al., 1995) and PH (Harlan et al., 1994) domains of several proteins have also been shown to bind PI 4,5-P_2. Thus, it is apparent from these studies that PI 4,5-P_2 plays a pivotal role in the functions of several proteins that are involved in signal transduction. Recent studies indicate that novel lipids (i.e., PI 3,4-P_2 and PI 3,4,5-P_3) may also bind to profilin and gelsolin, and actually may exhibit a similar function. The stimulation of PI 3-kinase-mediated phos-

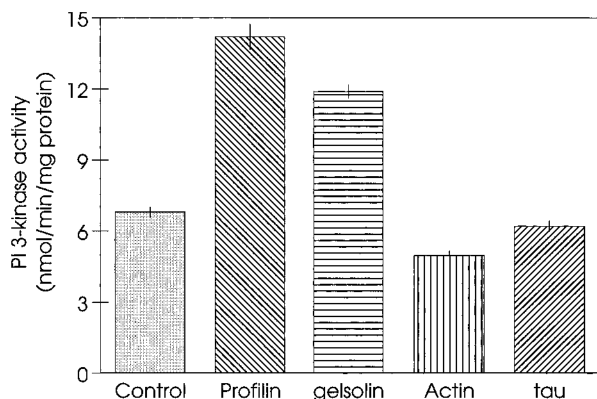


FIGURE 5: Effect of gelsolin, actin, and tau on PI 3-kinase activity. Different cytoskeletal proteins, 5 μM gelsolin, 5.7 μM actin, or 5.7 μM tau were incubated with 200 μM PS/PI 4,5-P_2 (1:1) for 30 min at room temperature, and lipid kinase activity was measured as described in Figure 1. Controls were run in the absence of cytoskeletal proteins. The results are expressed as Mean ± SE of four determinations.

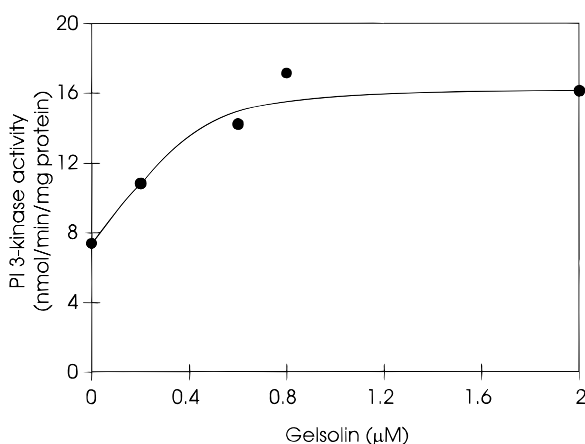


FIGURE 6: Effect of various concentrations of gelsolin on PI 3-kinase activity. Different concentrations of gelsolin were pre-incubated with 200 μM PS/PI 4,5-P_2 (1:1) for 30 min at room temperature, and lipid kinase activity was measured as described in Figure 1. The data are representative of two experiments. The experimental error for all data points is less than 10%.

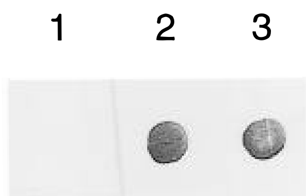


FIGURE 7: Binding of the P85 subunit of PI 3-kinase to profilin. Actin (2 μg) (lane 1), profilin (1 μg) (lane 2), and actin (2 μg) + profilin (1 μg) (lane 3) were spotted on the nitrocellulose membrane. Binding of p85-GST (fusion protein) was measured, as described in Experimental Procedures, using a monoclonal antibody raised against GST.

phorylation of phosphoinositides by profilin and gelsolin in the present study suggests that binding of phosphoinositides to profilin/gelsolin may be important for (i) the dissociation of actin from its complex with profilin/gelsolin and (ii) the rapid conversion of phosphoinositides into 3-OH phosphorylated phosphoinositides.

The stimulation of PI 3-kinase-mediated phosphorylation of phosphoinositides by profilin may not be attributed entirely to its association to phosphoinositides, because profilin was found to stimulate the phosphorylation of PI also, which does not have any affinity for profilin at physiological salt concentrations (Lassing & Lindberg, 1985). These results indicate that profilin may interact directly with PI 3-kinase. In fact, the binding of profilin to the 85-kDa subunit of PI 3-kinase suggests that profilin may be affecting the activity of kinase by its direct association to the regulatory p85 subunit of PI 3-kinase. Similarly, α-actinin (a cytoskeletal protein) has been shown to bind to the p85 subunit of PI 3-kinase (Shibasaki et al., 1994). We do not know at present the site of profilin binding to the 85-kDa subunit. However, our results on pyrene-labeled actin polymerization indicate that PI 3-kinase does not interfere with the binding of actin to profilin. This suggests that actin and PI 3-kinase do not share a common binding site on profilin.

Both profilin and gelsolin stimulated PI 3-kinase-mediated phosphorylation of PI 4,5-P_2 in a concentration-dependent manner, with maximal stimulation at 7.5 μM profilin and 0.8 μM gelsolin. The free profilin concentration in several cell types has been reported to be 3−10 μM (Lind et al., 1987). Therefore, the effect of profilin observed in our studies is in its physiological range. The profilin- and gelsolin-mediated stimulation of lipid kinase activity of PI 3-kinase appears to be specific because other cytoskeletal proteins such as tau or actin did not affect the lipid kinase activity of PI 3-kinase. It remains to be studied whether other phosphoinositide-binding proteins such as PH or SH2 domains affect PI 3-kinase activity. Profilin was found to affect only the lipid kinase activity of PI 3-kinase, not its protein kinase activity. Therefore, the binding of profilin to PI 3-kinase or phosphoinositides may not affect the other functions of PI 3-kinase such as phosphorylation of IRS-1.

Kinetic studies of PI 3-kinase-mediated phosphorylation of PI 4,5-P_2 suggest that profilin does not affect the affinities of the enzyme for ATP or PI 4,5-P_2. However, it increases the V_{max} of PI 3-kinase, which suggests that PI 4,5-P_2 bound to profilin or gelsolin may increase the accessibility of substrate, i.e., PI 4,5-P_2 to the enzyme. We ruled out the possibility that aggregation of PI 4,5-P_2 vesicles by profilin plays a role in profilin-mediated stimulation of PI 3-kinase activity because profilin could not induce the aggregation of vesicles containing PI 4,5-P_2. Bridging of the vesicles is an important step for the aggregation to occur (Papahadjopoulos et al., 1978). Our results suggest that binding of profilin to PI 4,5-P_2 vesicles does not bridge the two vesicles together. One may argue that the effect of profilin or gelsolin on PI 3-kinase activity may be due to the modulation of phosphoinositides/Mg^{2+} interaction by profilin or gelsolin. Janmey and Stossel (1989) reported that Mg^{2+} reacts differently to PI 4-P vesicles and PI 4,5-P_2 micelles, and that Mg^{2+} abolishes the inhibition of gelsolin by PI 4,5-P_2 micelles but not PI 4-P vesicles. However, both these lipids, PI 4-P and PI 4,5-P_2, when mixed with equal amount of PI, behave similarly in response to Mg^{2+}. In our studies, we have used mixed liposomes of PS/phosphoinositides (1/1, mol/mol). Therefore, profilin may not have affected the Mg^{2+}/phosphoinositide interaction.

We and others have presented evidence earlier that the profilin/phosphoinositides complex is phosphorylated by PKC in the presence of calcium but independent of phosphatidylserine and diacylglycerol (Singh et al., in press; Hanson et al., 1988). On the basis of these reports and the results of the present study, we suggest that profilin may

have a novel role in cell signalling.

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