Journal of Cellular Biochemistry 37:255–267 (1988)
Signal Transduction in Cytoplasmic Organization
and Cell Motility 173–185

Specificity of the Interaction Between Phosphatidylinositol 4,5-bisphosphate and the Profilin:Actin Complex

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Profilactin, the profilin:actin complex, which is present in large amounts in extracts of many types of eukaryotic cells, appears to serve as the precursor of microfilaments. It was reported recently that profilactin interacts specifically with phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) (Lassing and Lindberg: *Nature* 314:472–474, 1985.) The present paper describes in detail the behaviour of profilactin and profilin in the presence of different types of phospholipids and neutral lipids under different conditions. PtdIns(4,5)P_2 is the only phospholipid found so far which in the presence of 80 mM KCl and at Ca^{2+} concentrations below 10^{-5} M effectively dissociates profilactin with the resulting polymerization of the actin. Phosphatidylinositol 4-monophosphate exhibits some activity but phosphatidylinositol is inactive. Both calf spleen profilin and profilin from human platelets form stable complexes with PtdIns(4,5)P_2 micelles. PtdIns(4,5)P_2 is active also when incorporated together with other phospholipids in mixed vesicles.

Key words: profilactin, profilin, phospholipids, microfilament formation

In many types of eukaryotic cells there is a weave of highly organized microfilaments (actin filaments + associated proteins) in the immediate vicinity of the plasma membrane [1,2]. The dynamic morphological transformations often seen in the cell surface—e.g., movements of membrane lamellae and microspikes—appear to depend on assembly, translocation, and disassembly of the microfilaments building up the surface structures. Specific ligand/receptor interactions induce actin polymerization close to the

Abbreviations used: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′,-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; DTT, dithiotreitol; SDS, sodium dodecylsulfate; Tris, tris(hydroxy) aminomethane; PtdE, phosphatidylethanolamine; $PtdC_{dp}$, phosphatidylcholine (dipalmitoyl); $PtdC_{da}$, phosphatidylcholine (diarachidoyl); PtdS,phosphatidylserine; PtdG, phosphatidylglycerol; PhA, phosphatidic acid; $PtdIns_{sb}$, phosphatidylinositol containing primarily linoleic and palmitic acids; $PtdIns_{pl}$, phosphatidylinositol part of which has arachidonic acid in the sn-2 position; PtdIns (4)P, phosphatidylinostol 4-monophosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate.

Received March 20, 1987; accepted September 25, 1987.

plasma membrane [3–10]. The actin for this filament formation seems to be derived from profilactin [11]. We have recently shown [12] that profilactin specifically interacts with PtdIns(4,5)P_2 and that the interaction results in dissociation of the complex. Under physiological salt conditions the released actin then assembles into filaments.

This paper gives a detailed account of our observations concerning the effect of different phospholipids on profilactin. It is shown that PtdIns(4,5)P_2 in micellar form as well as in lipid vesicles together with other phospholipids acts specifically by binding to profilin.

The realization that there is a close correlation between receptor-mediated activation of phosphatidylinositol cycle and induction of microfilament formation [12] suggests the possibility that the PI cycle is of central importance to the regulation of microfilament-based motility.

MATERIALS AND METHODS

The phospholipids phosphatidylethanolamine (PtdE), phosphatidylcholine (dipalmitoyl) ($PtdC_{dp}$), phosphatidecholine (diarachidoyl)($PtdC_{da}$), phosphatidylserine (PtdS), phosphatideglycerol (PtdG), phosphatidic acid (PhA), PtdIns(4,5)P_2, PtdIns(4)P, and phosphatidylinositol from soy bean ($PtdIns_{sb}$; containing primarily linoleic and palmitic acids) were purchased from Sigma. Phosphatidylinositol from pig liver ($PtdIns_{pi}$; part of which has arachidonic acid in the sn-2 position) was from Serdary (London, Canada). This material was further purified by neomycin affinity chromatography [13]. PtdIns(4)P and PtdIns(4,5)P_2 were also isolated from calf brain by neomycin-affinity chromatography [13]. The 1,2-diolein was from Sigma. Oleyl 2-acetyl-diglyceride and IP_3 were generous gifts from Dr. Roger Sundler (Lund, Sweden). The PtdIns L-α-1-stearoyl-2-arachidonyl (arachidonyl-1-^{14}C) (10–30 mCi/mmol) and ^{14}PtdC L-α-palmitoyl-2-arachidonyl (arachidonyl-1-^{14}C) were from New England Nuclear.

Deoxyribonuclease I (DNAase I), ribonuclease A (RNAase A), soy bean trypsin inhibitor, cytochrome c, and lysozyme were products of Sigma. Heparin (average M_r 12,000–15,000) and Fragmin (a 14–20 sugar residue fragment of heparin; M_r 4,000–6,000) were generous gifts from KabiVitrum, Sweden.

Lipid Analyses

The purity of the lipids was assessed by thin-layer chromatography according to Palmer [13]. For this phospholipid samples were spotted on silica gel plates (HPTLC Fertigplatten, Kieselgel 60, Merck) precoated with 1% potassium oxalate in methanol:water (2:3) and activated for 30 min at 120°C before use. The plates were developed for 60 min with chloroform:acetone:methanol:acetic acid:water (40:15:13:12:8), dried, and stained with iodine vapor and 3% copper acetate in 8% H_3PO_4 at 180°C for 10 min.

The PtdE, PtdG, and PtdC were delivered as stock solutions (in chloroform) of known concentration. PtdS was obtained as a powder which was weighed out and dissolved in chloroform. Stock solutions of PtdIns, PtdIns(4)P, PtdIns(4,5)P_2, and PhA were made in chloroform and the concentration of phospholipid was determined by analyzing the phosphate content according to Hess and Derr [14]. The lipid content in proteolipid complexes isolated by gel chromatography was analyzed by extracting the lipids with 2 volumes of chloroform(C):methanol(M):2.4 M HCl (1:2:0.1). The chloroform phase was dried and dissolved in 50 μl of C:M (1:1), and the lipids were analyzed according to Palmer [13].

Lipid Vesicles and Micelles

Lipid vesicles and micelles were made by drying known amounts of the lipids either under nitrogen or under vacuum in a Speed Vac centrifuge (Savant Instruments, Hicksville, New York) and sonicating the dried material in 100 μl of G buffer. Lipid particles generated by sonication on ice and under nitrogen in a ultrasonicator-type A350 G (Ultrasonic Ltd., England) were used in the experiments of Figs. 1–3A. In the other experiments sonication was performed at 80°C by using a Sonicor water bath (Sonicor Instruments Corp., Copiaque, New York) [15].

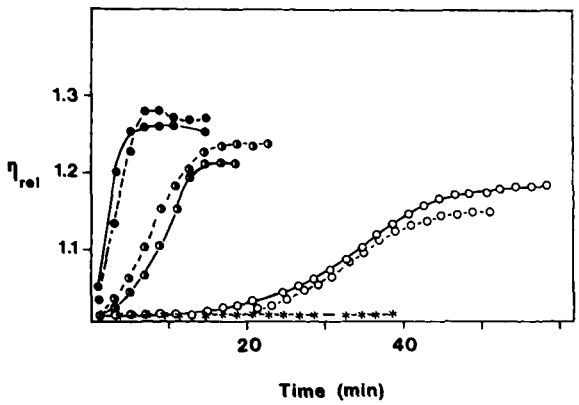

Fig. 1. Behaviour of the two isoforms of profilactin treated with PtdIns under low salt/high Ca^{2+} conditions and then exposed to polymerizing conditions. Profilactin (PA)(0.4 mg ml^{-1}) containing either β- or γ-actin was incubated with PtdIns vesicles (0.8 mg ml^{-1}) in low salt/high Ca^{2+} conditions as described in the text, after which either KCl or $MgCl_2$ was added to induce polymerization. Symbols: *-* PA preincubated without PtdIns and then with 80 mM KCl in the viscometer; ○ - ○ and ○—○, PA_β and Pa_γ respectively with PtdIns and then with 80 mM KCl; ◑ - ◑ and ◑—◑ Pa_β and PA_γ without PtdIns and then with 2 mM $MgCl_2$; ● - ● and ●—●, PA_β and PA_γ respectively with PtdIns and then with 2 mM $MgCl_2$.

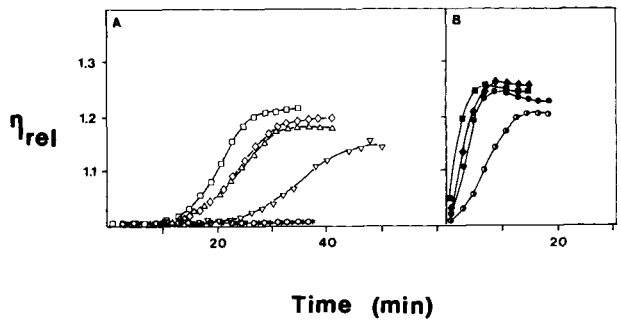

Fig. 2. Behaviour of profilactin$_\beta$ preincubated with different phospholipids under low salt/high Ca^{2+} conditions and then exposed to polymerizing conditions. Profilactin$_\beta$ (0.4 mg ml^{-1}) was preincubated with the phospholipid particles (0.8 mg ml^{-1}) after which polymerizing salt was added (see text). **Panel A**: PA_β preincubated with PtdE (○); PtdC (*); PtdG (▽); PtdS (△); PtdA (◇); or PtdIns(4,5)P_2 (□). Polymerization was initiated by the addition of 80 mM KCl. **Panel B**: PA_β without phospholipid (◑), with PtdIns (●); PtdA (◆); and PtdIns(4,5)P_2 (■). Here polymerization was initiated with 2 mM $MgCl_2$. In all cases zero time marks the addition of the polymerizing salt.

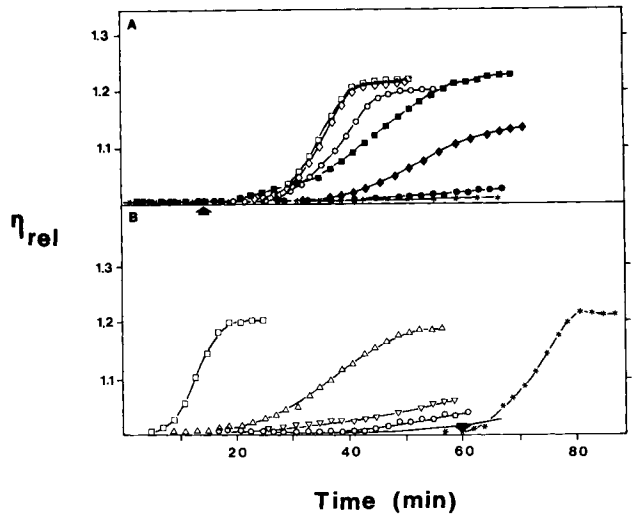

Fig. 3. Polymerization of actin from profilactin$_\beta$ preincubated with phospholipids in low salt/high Ca^{2+} in comparison with the polymerization seen after exposure of the profilactin to phospholipids in high salt/high Ca^{2+} or high salt/low Ca^{2+}. **Panel A**: Profilactin (0.4 mg ml^{-1}) preincubated in low salt/high Ca^{2+} (see text) with PtdIns(4,5)P_2 (□), PtdA (◇), and PtdS (○). Here KCl (80 mM) was added at the time indicated by the arrow. In the other cases profilactin plus either PtdIns(4,5)P_2 (■) or PtdA (◆) or PtdS (●) was incubated from time zero with 80 mM KCl. Concentration of phospholipids was 0.8 mg ml^{-1}. **Panel B**: PA_β incubated with PtdIns(4,5)P_2 (□), PtdIns(4)P (△), PtdA (—), PtdIns$_{pl}$ (▽), or PtdS (○). Here the buffer contained 5 mM potassium phosphate, pH 7.6, 80 mM KCl, 0.5 mM ATP, 0.5 mM DTT, 10 μM EDTA, 0.1 mM $CaCl_2$, and 0.1 mM EGTA giving a final Ca^{2+} concentration of 10^{-6} M. Profilactin alone in 80 mM KCl (*-*) remained stable until $MgCl_2$ was added (arrow).

Preparation of Proteins

Profilactin was prepared from calf spleen essentially as described by Carlsson et al. [16], and the two isoforms containing β- and γ-actin respectively were separated by chromatography on hydroxylapatite [17]. All profilactin preparations were rechromatographed on a Sephadex G-100 superfine (Pharmacia, Sweden) equilibrated with 5 mM potassium phosphate buffer pH 7.6, 0.2 mM $CaCl_2$, 0.5 mM ATP, 0.5 mM dithiotreitol (DTT), and 0.03% NaN_3 as described by Larsson [18]. This removes the profilactin-stabilizing factor which otherwise contaminates the preparation [18,19]. Calf spleen profilin with an intact C-terminus and spleen actin was prepared according to Malm et al. [19]. Profilin from human platelets, generously provided by Dr. Francis Markey, was purified essentially as described earlier [20].

Analysis of Profilin:Lipid Mixtures by Gel Chromatography

Profilin:lipid mixtures were analyzed on Superose 6B columns (Pharmacia, Sweden) equilibrated with 5 mM potassium phosphate pH 7.6, 80 mM KCl, 0.1 mM $CaCl_2$, 10 μM EDTA, 0.5 mM DTT, 0.03% NaN_3, and 0.1 mM EGTA, giving a final Ca^{2+} concentration of about 10^{-6} M. The flow rate was 20 ml per hour and 0.6-ml fractions were collected. The absorbance of the effluent at 280 nm was either monitored automatically by using a LKB Uvicord or measured manually by using a Zeiss PMQ III spectrophotometer. Radioactivity was measured in an Intertechnique scintillation counter

by taking 0.3 ml of every other fraction and adding 10 ml of toluene:methanol (1:1) containing PPO/POPOP (4 g and 0.5 g respectively per 1,000 ml).

Assay of Profilactin-Destabilizing Effects of Lipids by Viscometry

The dissociation constant (K_d) of the profilin:actin complex is $<4 \times 10^{-8}$ M in 50 mM KCl, 0.1 mM $CaCl_2$ 0.5 mM ATP, 0.5 mM DTT, 10 μM EDTA, 5 mM potassium phosphate, pH 7.6 (25°C) (no Mg^{2+}) and there is no filament formation even after prolonged incubations (hours) as shown by Larsson [18]. These conditions were used here to investigate the possible destabilizing effects of different lipids on the profilactin complex by viscometry, under the assumption that destabilization, releasing enough actin from profilactin to exceed the critical concentration for actin polymerization, would result in filament formation and increased viscosity of the sample. The profilactin-lipid mixtures were incubated in a Cannon-Manning viscometer at 35°C (flow times 45 sec), and the viscosities of the mixtures were measured with 2-min intervals for up to 2.5 hr. In cases where polymerization had not occurred at the end of the incubation, the status of the profilactin was checked by adding $MgCl_2$ to 2 mM. This increases the K_d of the profilin:actin complex to 4×10^{-7} M [18] and results in the spontaneous formation of actin filaments from a large fraction of the profilactin.

Gel Electrophoresis

Proteins were analyzed by electrophoresis on SDS-15% polyacrylamide gels [21]. When necessary the gels were scanned with an LKB 2202 Ultroscan after staining with Coomassie blue.

Protein concentrations were determined spectrophotometrically by using an $E^{1\%}_{1\ cm}$ at 290 nm for actin of 6.3 and $E^{1\%}_{1\ cm}$ at 280 nm of 11.0 for profilactin and 12.0 for profilin.

RESULTS

The Effect of Lipids on Profilactin at Low Salt/High Ca^{2+}

To test the effect of lipids on the stability of profilactin under low salt/high Ca^{2+} conditions, the protein (0.4 mg ml^{-1}) was first incubated with the lipid particles (0.8 mg ml^{-1}) in 5 mM potassium phosphate buffer pH 7.6, 0.1 mM $CaCl_2$, 10 μM EDTA, 0.5 mM ATP, 0.5 mM DTT for 15 min, after which KCl was added to 50 mM to induce filament formation. Preincubation of profilactin under these conditions with the anionic phospholipids $PtdIns_{sb}$ (Fig. 1), and PtdG, PhA, PtdS, and $PtdIns(4,5)P_2$ (Fig. 2A) resulted in filament formation after the salt addition. The $t_{1/2}$ of the polymerizations (counting from the addition of salt) were 35, 34, 23, 23, and 21 min, respectively. In the case of $PtdIns_{sb}$, experiments were performed with both $profilactin_{\beta}$ and $profilactin_{\gamma}$, and as shown in Figure 1 the two isoforms of profilactin gave closely similar results.

The zwitterionic phospholipids PtdE and PtdC, on the other hand, did not have any effect on profilactin (Fig. 2A), and the neutral lipids 1,2-diolein and oleyl 2-acetyl diglyceride were also inactive (not shown).

The Effect of Lipids in the Presence of Mg^{2+}

In the presence of 2 mM $MgCl_2$ spontaneous filament formation due to the increase in K_d of the profilin:actin complex (see Materials and Methods) occurred with a $t_{1/2}$ of about 8 min (Fig. 1). When profilactin was preincubated with different anionic phos-

pholipids in low salt buffer and then exposed to 2 mM $MgCl_2$ the $t_{1/2}$ was shortened to 2–3 min (Figs. 1, 2B). Again there was no significant difference between the two isoforms of profilactin, and the subsequent experiments were therefore performed only with profilactin$_\beta$.

Effects of Lipids on Profilactin at Increased Salt Concentrations and Varying Ca^{2+} Concentrations

The effect of preincubating profilactin with anionic phospholipids at low salt/high Ca^{2+} concentrations on the stability of the complex in 80 mM KCl was compared with the effect seen when the components were mixed in 80 mM KCl and 0.1 mM Ca^{2+} from the beginning of the experiment. The examples given in Figure 3A clearly show that adding salt at the time of mixing prolonged the lag phase in the polymerization with both PtdIns(4,5)P_2 and PhA, and with PtdS the activity was almost completely suppressed.

If the potassium chloride concentration of the initial mixture was kept at 80 mM and the Ca^{2+} concentration was lowered to 10^{-6} M PhA, PtdS and PtdIns caused only a slow polymerization (Fig. 3B). The polyphosphoinositides, PtdIns(4)P and PtdIns(4,5)P_2, on the other hand, exhibited an increased activity at lower Ca^{2+} concentrations with PtdIns(4,5)P_2 being the most active of the two (Fig. 3B).

The Ca^{2+} dependence of the interaction between profilactin and the polyphosphoinositides is illustrated in Figure 4A and B. In the case of PtdIns(4,5)P_2 there was a pronounced increase in the activity when the Ca^{2+} concentration was lowered from 10^{-4} M to 10^{-5} M (80 mM KCl) (Fig. 4A). Also with PtdIns(4)P an increase in the activity was observed at decreasing Ca^{2+} concentrations (Fig. 4B), but here the increase was more gradual. At all Ca^{2+} concentrations PtdIns(4)-monophosphate was less active than the -bisphosphate.

The kinetics of the polymerization of actin from profilactin in the presence of PtdIns(4,5)P_2 ($t_{1/2}$ = 12.5 min; 80 mM KCl, 10^{-6} M Ca^{2+}; Fig. 4A) were closely similar to those of actin alone ($t_{1/2}$ = 9.5 min) and of actin in the presence of PtdIns(4,5)P_2

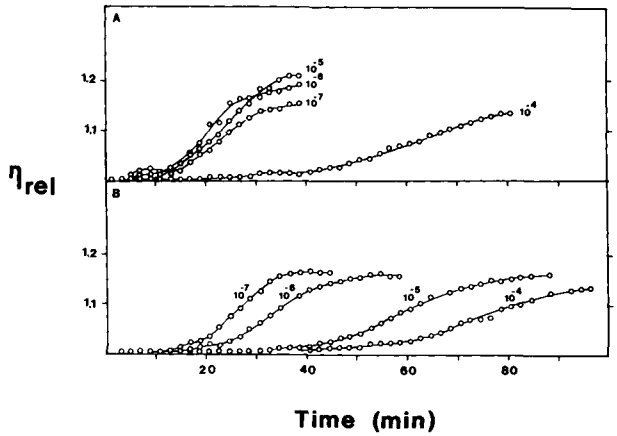

Fig. 4. Effect of Ca^{2+} on the interaction between polyphosphoinositides and profilactin. Profilactin$_\beta$ was incubated with PtdIns(4,5)P_2, 0.2 mg ml^{-1} (**panel A**), or PtdIns(4)P, 0.2 mg ml^{-1} (**panel B**), in buffers containing 5 mM potassium phosphate, pH 7.6, 80 mM KCl, 10 μM EDTA, 0.5 mM ATP, 0.5 mM DTT, 0.1 mM $CaCl_2$, and EGTA to give the Ca^{2+} concentrations (M) indicated in the figure.

($t_{1/2}$ = 11 min) (data not shown). This suggested that PtdIns(4,5)P_2 somehow eliminated the polymerization-inhibiting effect of profilin.

Complex Formation Between Profilin and Phospholipids

Evidence that PtdIns(4,5)P_2 dissociates profilactin and forms a complex with profilin in low salt/high Ca^{2+} buffer was obtained by chromatography of the reaction products on Sephacryl 400 as reported recently [12]. However, preincubation of profilactin under those conditions with any of the anionic phospholipids facilitated actin filament formation on subsequent salt addition (Figs. 1, 2). Thus in the low salt/high Ca^{2+} conditions profilactin appeared to interact also with lipids other than PtdIns(4,5)P_2. Chromatographic analysis of profilin mixed with PtdIns, PhA, and PtdC respectively demonstrated that complex formation indeed occurred with PhA and PtdIns under low salt conditions but not with PtdC, and that PhA was the most active of these two anionic phospholipids (data not shown). It was important therefore to test the specificity of the phospholipid effect under conditions which according to the polymerization assay were the most stringent, i.e., 80 mM KCl/10^{-6} M Ca^{2+} (Fig. 2B).

Since the products formed in the interaction between profilactin and the phospholipids active in 80 mM KCl/10^{-6} M Ca^{2+} are not readily analyzed by gel chromatography due to polymerization of the actin, the specificity of the phospholipid effect under these conditions was analyzed with the use of isolated calf spleen profilin. The somewhat less basic profilin from human platelets was analyzed in parallel. As shown in Figure 5D and H, both types of profilin formed stable complexes with PtdIns(4,5)P_2, whereas in the case of PtdIns(4)P (Fig. 5C, G) only small amounts of either profilin were recovered in positions indicating complex formation. With PtdIns no stable complex was observed (Fig. 5B, F).

Interactions With Mixed Vesicles

In aqueous solutions PtdIns(4,5)P_2 forms micelles [22,23].These particles have a very high density of negative charge on their surface due to the phosphates on the inositol ring. To investigate whether profilin would interact with particles whose surface had a lower density of PtdIns(4,5)P_2 than the micelles, this phospholipid was incorporated together with other phospholipids into bilayered lipid vesicles and their effect on profilin was then analyzed. Figure 6A shows that lipid vesicles containing PtdE, PtdS, PtdG, and PtdIns (no PtdIns(4,5)P_2) did not affect the chromatographic behaviour of calf spleen profilin. When the vesicles contained PtdIns(4,5)P_2, on the other hand, a large fraction of both profilin and the lipid changed chromatographic behaviour and were eluted together, suggesting complex formation (Fig. 5B).

Analysis of the lipid recovered from the proteolipid particles (see Materials and Methods) demonstrated that it had the same relative composition as the original sample, strongly suggesting that profilin had bound to PtdIns(4,5)P_2 present in mixed vesicles (data not shown). The proportion of the PtdIns(4,5)P_2 available to interact with profilin in the mixed vesicles was assessed by determining the proportion of the monoesterified phosphate which could be released by digestion with alkaline phosphatase. This analysis showed that about 50–60% of the PtdIns(4,5)P_2 was available to the enzyme and thus presumably available to interaction with profilin.

Vesicles formed with PtdIns(4,5)P_2, PtdE, and PtdC did not bind profilin as shown in Figure 6C. Thus the large, positively charged head group of PtdC apparently interferes with PtdIns(4,5)P_2 in its interaction with profilin.

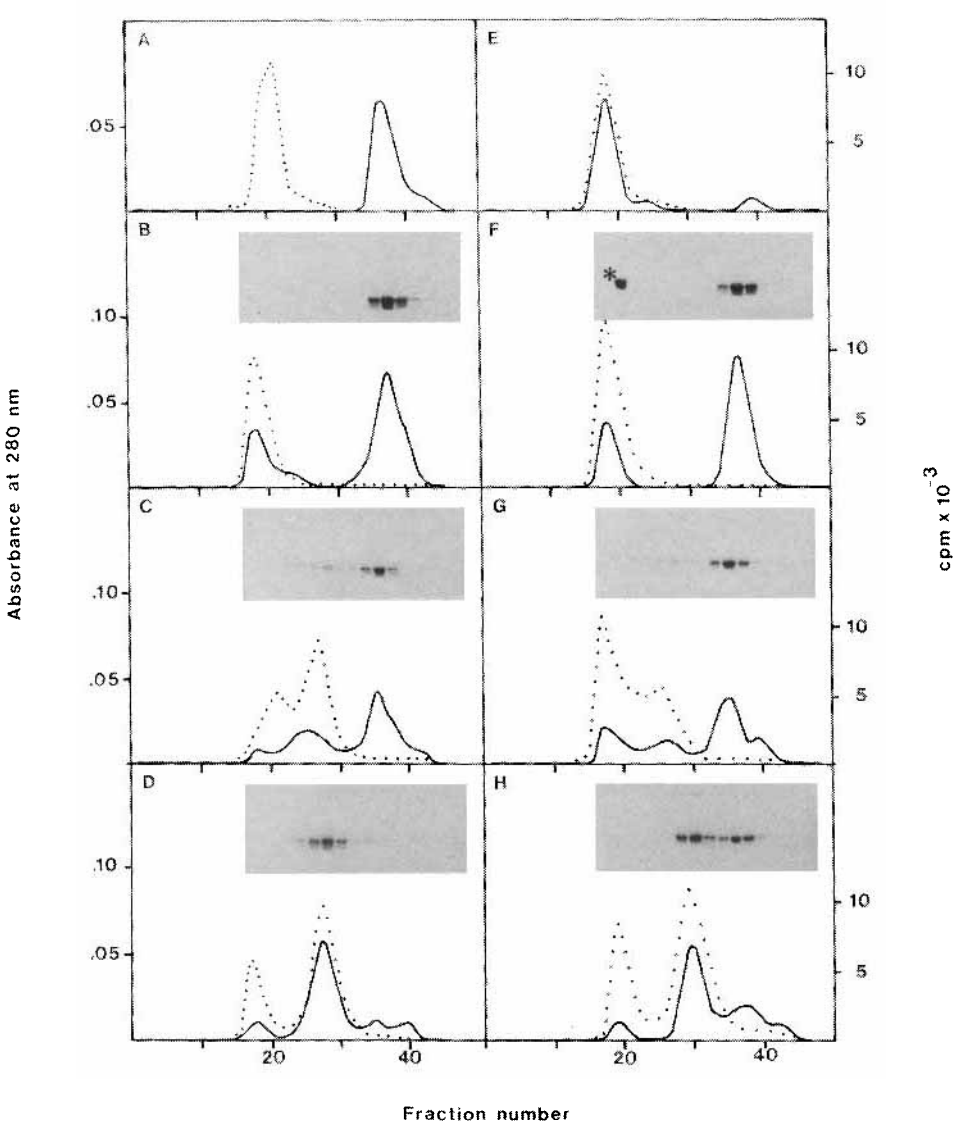

Fig. 5. The effect of phosphoinositides on the chromatographic behaviour of profilin from calf spleen and human platelets. Samples of profilin (0.2 mg ml^{-1}) were incubated with phospholipid particles (0.4 mg, with ^{14}C-PtdIns added as marker) for 15 min at room temperature and then analyzed by gel chromatography on Superose 6B as described in Materials and Methods. Calf spleen profilin and human platelet profilin respectively were run with PtdIns (**B** and **F**), PtdIns(4)P (**C** and **G**), and PtdIns(4,5)P_2 (**D** and **H**). Panel **A** illustrates two separate experiments: one in which PtdIns(4,5)P_2 micelles were chromatographed alone (···, left peak) and one with calf spleen profilin alone (—, right peak), and panel **E** shows the elution profile obtained with PtdIns vesicles alone. The fractions were analyzed for radioactivity (···) and absorbance at 280 nm (—). Insets show the analyses of the fractions by SDS-polyacrylamide gel electrophoresis. The band at position * is a profilin marker.

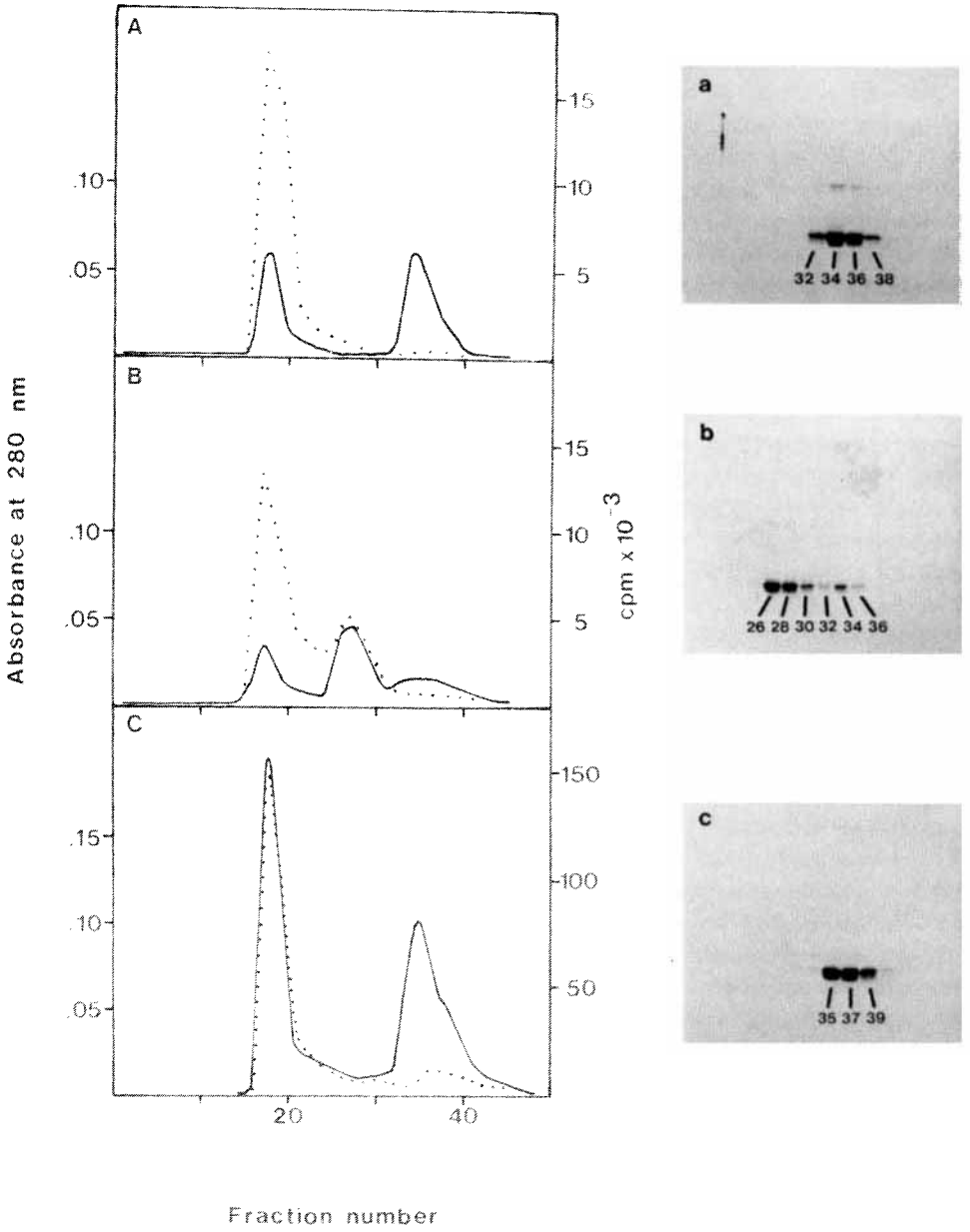

Fig. 6. Analysis of mixtures of calf spleen profilin and mixed lipid vesicles by gel chromatography. Profilin samples (0.2 mg) were mixed with 0.4 mg of mixed vesicles. **Panel A**: Profilin plus mixed vesicles containing PtdE, PtdS, PtdG, and PtdIns (weight ratios: 46:23:23:8). **Panel B**: Profilin plus vesicles containing PtdE (PE), PtdS (PS), PtdG (PG), PtdIns (PI), PtdIns(4,5)P_2 (PIP_2) (weight ratios: 30:15:15:5:35). **Panel C**: Profilin plus vesicles with PtdE, PtdC, PtdIns(4,5)P_2 (weight ratios: 33:33:34). **Panels a–c** show the analyses of the fractions (as indicated) by SDS-polyacrylamide gel electrophoresis.

Additional Controls

To further test the specificity of the PtdIns(4,5)P_2/profilin interaction, several different proteins were incubated with PtdIns(4,5)P_2 micelles in 80 mM KCl/10^{-6} M Ca^{2+}, and the mixtures were analyzed by chromatography on Superose 6B. There was no detectable interaction with either deoxyribonuclease I (M_r 31,000, pI 4,5), ribonuclease A (M_r 13,800, pI 7.8), or soy bean trypsin inhibitor (M_r 20,100, pI 4.5). Cytochrome c (M_r 12,200, pI 10.8), however, appeared to interact with PtdIns(4,5)P_2 micelles, forming a complex which had a chromatographic behaviour similar to that of the profilin:PtdIns(4,5)P_2 complex. The highly basic protein lysozyme (M_r 13,900, pI 11.0) caused precipitation of all anionic phospholipids tested, regardless of the conditions (data not shown).

The effect of $InsP_3$ (the primary product formed by hydrolysis of PtdIns(4,5)P_2 in response to certain ligand/receptor-interactions, see Discussion) on profilactin and on the profilactin-dissociating activity of PtdIns(4,5)P_2 was also tested. For this profilactin was incubated in the 80 mM KCl/10^{-6} M Ca^{2+} buffer with a 25-fold molar excess of $InsP_3$ and the viscosity of the sample was followed for 70 min. Under these conditions there was no increase in the viscosity of the sample. That the status of the profilactin had not changed during the incubation was indicated by the fact that the actin was fully capable of forming filaments on addition of 2 mM $MgCl_2$ after the 70-min incubation with $InsP_3$ (data not shown).

When profilactin was incubated for 5 min with a 25-fold molar excess of $InsP_3$ and then exposed to PtdIns(4,5)P_2 micelles, filament formation occurred with a time course closely similar to that seen when the incubation with $InsP_3$ was omitted (data not shown). Thus $InsP_3$ could not by itself cause dissociation of profilactin, nor did it seem to interfere with the PtdIns(4,5)P_2 effect on profilactin.

Since PtdIns(4,5)P_2 forms highly negatively charged particles it was important to test whether polyanions in general bind profilin and cause dissociation of profilactin the protein complex. For this profilactin (0.4 mg ml^{-1}) was incubated with the sulphated polysaccharide heparin (0.4 and 8 mg ml^{-1} were tested) as well as a fragmented heparin product, Fragmin (0.8 mg ml^{-1}) in the 80 mM KCl/10^{-6} M Ca^{2+} buffer and analyzed by viscosimetry. However, in neither case was there any increase in the viscosity, and the induction of filament formation by Mg^{2+} at the end of the incubation ensured that the actin remained native during the whole time period (data not shown).

DISCUSSION

At near-physiological levels of potassium chloride (80 mM) and Ca^{2+} (10^{-6} M) the polyphosphoinositides were the only phospholipids which remained highly active in binding to profilin and in dissociating the profilactin complex. The increase in activity of the inositides with increasing phosphorylation of the inositol ring demonstrates the importance of the phosphate groups in the inositide/profilin interaction. The relatively strong interference with this interaction caused by the addition of Ca^{2+} points in the same direction, since chelation of divalent cations by these phospholipids most likely involves the phosphate group(s) on the inositol ring [24].

If there is a specific binding site for PtdIns(4,5)P_2 on profilin one would expect $InsP_3$ by itself either to affect the stability of profilactin or to interfere with the interaction between profilin and the phospholipid. However, $InsP_3$ added in a 25-fold excess over profilactin affected neither the stability of profilactin nor the effect of PtdIns(4,5)P_2 on

the complex, suggesting that the binding of profilin is qualitatively different when the phosphorylated inositol is present in the form of PtdIns(4,5)P_2 on the surface of micelles or mixed vesicles. It is possible that there is a secondary interaction between profilin and the hydrophobic part of the phospholipid or that profilin has to interact with the phosphorylated inositols of at least two adjacent phospholipid molecules to form a stable complex from which actin is dissociated.

Experiments aimed at determining the stoichiometry of the interaction indicated that about 10 mol of PtdIns(4,5)P_2 is needed to dissociate 1 mol of profilactin [12]. Considering that there might not be space enough to accommodate more than about eight molecules of profilin (profilactin) per phospholipid micelle (M_r of 93,000, packing number of 82 [22]) these results suggest the possibility of a specific binding site for PtdIns(4,5)P_2 on profilin.

When PtdIns(4,5)P_2 was inserted into mixed vesicles, it still bound profilin and caused dissociation of the profilactin complex. If steric hindrance were an important factor limiting the binding of profilactin to micelles, the mixed vesicles (where the PtdIns(4,5)P_2 would be more widely spaced than in the micelles) should be able to bind more profilactin per PtdIns(4,5)P_2. However, to resolve this question a more sensitive assay, preferably measuring the initial interaction between the lipid vesicles and profilactin, has to be developed.

The inhibition of the activity of PtdIns(4,5)P_2 by PtdC intermingled with PtdE and PtdIns(4,5)P_2 in mixed vesicles may suggest that the large headgroup of PtdC with its quartenary amine somehow neutralizes the effect of the two phosphates of the inositol ring of PtdIns(4,5)P_2. Phosphatidylcholine occurs mostly in the outer leaflet of the bilayer of the plasma membrane [25], and it is of interest to note that platelets having an abnormally high PtdC in the inner leaflet of the bilayer of the plasma membrane are less reactive than normal platelets [26].

The specificity of the interaction between PtdIns(4,5)P_2 and profilin is also illustrated by the fact that polyanions in general did not dissociate profilactin. Heparin (or Fragmin) with its sulphated sugar residues did not destabilize profilactin in the high salt/low Ca^{2+} buffer. Profilactin binds to poly-U- and poly-A-Sepharose but is eluted in intact form at increased salt concentration (Lindberg, unpublished). It does not bind to phosphocellulose, whereas profilin does. Chromatography of profilin on this matrix separates intact profilin from a form which lacks one or two amino acids at the C-terminus [19]. In contrast to this, profilin does not bind to Mono-S (Pharmacia) where the active group is $-CH_2\text{-}SO_3^-$. Hydroxyapatite, a calcium phosphate mineral, has only a weak affinity for free profilin, but profilactin is adsorbed, and elution with a salt gradient separates the two isoforms, profilactin$_\beta$ and profilactin$_\gamma$ [17].

Positively charged agents like polylysine, spermidine, and putrescine have been shown by others to promote actin polymerization [27], but they do not trigger a fast polymerization of actin from profilactin (data not shown).

In the chromatographic analysis of the products formed by incubating profilactin with PtdIns(4,5)P_2 the major part of profilin was eluted together with the phospholipid particles ahead of the released actin [12]. Although most of the actin was eluted as free monomeric actin, about 10% of the total actin coeluted with the profilin:phospholipid complex. This suggests that PtdIns(4,5)P_2 binds to profilin in complex with actin and that this primary interaction is followed by a secondary change resulting in dissociation of the complex. Although both profilin and profilactin bind detergents [19], there is no direct evidence as yet that they interact with the hydrophobic parts of the lipid vesicles.

The role of profilactin and phospholipids in the formation and functioning of the microfilament system of the cell is still uncleaer. In vitro Mg^{2+} ions increase the dissociation constant of the profilin:actin complex to such an extent that filament formation occurs [19]. However, the lag phase of the polymerization of actin from profilactin seen after addition of Mg^{2+} is considerably longer than that observed with G-actin alone. Also, here PtdIns(4,5)P_2 shortens the lag phase so that the actin polymerizes as if profilin was not around, which is similar to the effect seen in the 80 mM KCl/10^{-6} M Ca^{2+} buffer.

In cell extracts profilactin is relatively stable even though Mg^{2+} ions are present [3], suggesting that there are factors other than profilin in the cell which contribute to the regulation of actin polymerization. As reported earlier [28,29] gelsolin and villin inhibit the Mg^{2+}-induced polymerization of actin from profilactin. These proteins bind to the fast-growing end (barbed end) of actin filaments and presumably to actin nuclei appearing in the solution, thereby preventing polymerization at this end [30,31]. Since profilactin apparently cannot provide actin for polymerization at the slow-growing (pointed) end [28,32], villin and gelsolin, by blocking barbed-end polymerization, cause an apparent stabilization of profilactin. Clearly induction of filaments in the cell has to employ mechanisms that are able to overcome both the effect of profilin and of proteins like gelsolin and villin. The observation that PtdIns(4,5)P_2 binds to gelsolin and thereby changes its reactivity toward actin [33] is of great interest in this context.

The findings reported here are of special interest since they imply a link between cell motility and mechanisms regulating cellular activities. They suggest that generation ofPtdIns(4,5)P_2 in the inner leaflet of the plasma membrane recruits profilactin, which then dissociates, providing actin for filament formation.

ACKNOWLEDGMENTS

We are most grateful to Anna-Lena Kullgren, Thomas Hult, and Janusz Garbalinsky for technical assistance. This work was supported by grants from the Swedish Cancer Society and the Swedish Natural Science Research Council.

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