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# Phosphatidylinositol Cycle and Its Possible Involvement in the Regulation of Cytoskeleton-Membrane Interactions

**Paul Burn**

*Department of Biology, University of California at San Diego, La Jolla, California 92093*

Receptor-mediated activation of many cells, including blood platelets, leads to changes at the cytoplasmic side of the membrane. In platelets, phospholipases, such as phospholipase C and phospholipase $A_2$, have been shown to become activated. From phospholipids they generate the second messengers diacylglycerol and inositol phosphate(s) and fatty acids, respectively. At the same time, actin polymerization and reorganization of actin filaments into bundles and networks occurs. Here, the association of lipids, radiolabeled either with saturated (palmitic acid) or unsaturated (arachidonic acid) fatty acids, with the cytoskeletons of resting and activated human blood platelets was studied. The relative binding of lipid components to the cytoskeleton of activated platelets labeled with palmitic acid is six times higher than that of platelets labeled with arachidonic acid. Analysis of lipids associated with isolated cytoskeletons of resting and activated platelets (labeled with palmitic acid) showed a 30-fold increase in the binding of labeled lipids to the cytoskeletal structures during activation. Both diacylglycerol and fatty acids were found to be associated with the cytoskeleton of activated platelets. Gel filtration, chromatofocusing, and immunoprecipitation studies demonstrated tight binding of these lipids to α-actinin. α-Actinin is one of the proteins that rapidly becomes associated with the cytoskeleton during platelet aggregation; it is also one of the molecules proposed to act as an actin-membrane linker. The results reported indicate a possible participation of α-actinin, fatty acids, and the phosphoinositide-derived second messenger diacylglycerol in the regulation of cytoskeleton-membrane interactions. Together with the results of others they suggest a possible involvement of the phosphatidylinositol cycle in the assembly of actin filaments and their anchoring to membranes.

**Key words: α-actinin, second messenger, diacylglycerol, fatty acids, platelets, actin-membrane association**

Abbreviations used: EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis (β-aminoethyl ether)-N,N, N′,N′-tetraacetic acid; PAGE, polyacrylamide gel electrophoresis; pI, isoelectric point; PRP, platelet-rich plasma; RCD, Ringer-citrate-dextrose buffer; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography.

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There are now many studies which support the notion that hydrolysis of inositol-containing phospholipids is a common response by many different kinds of cells to a wide variety of external stimuli [1–5]. Physiological activation of blood platelets by thrombin, for example, leads to the decrease of the phosphatidylinositol content in membranes within seconds [6–8]. The reaction, which is catalyzed by phospholipase C, generates two putative second messengers: diacylglycerol and inositol trisphosphate [1–5]. Inositol trisphosphate is released into the cytoplasm, where it functions in the mobilization of intracellular $Ca^{2+}$. Diacylglycerol operates within the plane of the membrane, where it has been shown to be important in the activation of the $Ca^{2+}$- and phospholipid-dependent protein kinase C [9,10].

Many blood platelet responses are thought to be regulated by cytoskeletal elements. The rapid shape change from discoid to spiny sphere platelets, for example, occurs when the actin filaments within the stimulated cells become reorganized into bundles and networks [11–13]. Studies of isolated cytoskeletons have shown that actin filaments become associated with other proteins during platelet activation and it is likely that these proteins are involved in the structural reorganization [11,14–16].

α-Actinin is one of the proteins whose association with the cytoskeleton is increased during the progressive stages of aggregation. As much as 10–15% of the total protein in isolated cytoskeletons of aggregated platelets is α-actinin [12,14,15]. It has been reported that α-actinin is present in blood platelets, both associated with membranes and in the cytoplasm [14,17–19]. Recent studies with model membranes demonstrated a specific interaction of α-actinin with fatty acids and diacylglycerol [20]. However, a difference in the interaction of saturated and nonsaturated lipids with α-actinin was observed. The formation of supramolecular complexes between α-actinin and actin in the presence of diacylglycerol and palmitic acid has also been reported [21,22]. These complexes displayed substructures similar to those of microfilament bundles in vivo. Similar protein-lipid complexes can also be formed in situ during the stimulation of blood platelet aggregation [21,22].

In this report the association of lipid components (radiolabeled with saturated or unsaturated fatty acids) with cytoskeletal structures of blood platelets is described. Lipid binding to the cytoskeleton of activated cells, as well as their binding to α-actinin, is reported. A possible involvement of the phosphatidylinositol cycle in cytoskeletal organization and in the regulation of cytoskeleton-membrane interactions is discussed.

## MATERIALS AND METHODS

### Lipids

Fatty acids, phospholipids, and diacylglycerol were purchased from Serdary Research Laboratories (London, Ontario) or from Sigma (Deisenhofen, West Germany). Radiolabeled fatty acids, [$^3$H]-palmitic acid, and [$^3$H]-arachidonic acid were obtained from New England Nuclear (Boston, MA).

### Platelet Preparation

Platelets were prepared by a modified procedure of Schmidt and Rasmussen [23]. Briefly, citrated blood was centrifuged at 150g for 20 min. The platelet-rich plasma (PRP) together with some erythrocytes was removed, and platelets were sedimented on the erythrocyte cushion. The plasma was removed and after resuspen-

sion of the cells in Ringer-citrate-dextrose buffer (RCD buffer: 1.6 mM $CaCl_2$, 3.9 mM KCl, 107.8 mM NaCl, 0.5% glucose, and 0.62% trisodium citrate, pH 7.4) the erythrocytes were centrigued down (150g for 20 min) and the platelet suspension was removed.

### Labeling of Platelets and Isolation of Cytoskeletons

The washed platelets ($3 \times 10^8$ cells/ml) were incubated at 23°C with either [$^3$H]-palmitic acid or [$^3$H]-arachidonic acid (30 $\mu$Ci/ml) in RCD buffer for 2 hr. The cells were then centrifuged at 10,000g for 4 min and the pellet was washed three times with RCD buffer. Triton X-100-insoluble cytoskeletons of resting and activated platelets were then isolated as described [14,18]. Thrombin-activation was performed with 1 unit $ml^{-1}$ (final concentration) at 37°C. After 20 sec a solution containing Triton X-100 and EGTA was added to the platelet suspension until the concentrations of Trition X-100 and EGTA were 1% and 5 mM, respectively. The suspension was stirred for a further 1 min, kept on ice for 30 min, and then centrifuged for 4 min at 10,000g. The supernatants were removed and the pellets washed twice with RCD buffer containing 1% Triton X-100 and 5 mM EGTA, and once with RCD buffer only.

### Immunoprecipitation

Isolated cytoskeletons were dissolved in 1% SDS, heated for 3 min at 95°C, and then centrifuged for 4 min at 10,000g. The supernatant fraction was diluted with TNET-buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100) to give a final concentration of 0.1% SDS. The diluted suspension was precleared with 100 $\mu$l of a 10% w/v suspension of glutaraldehyde-fixed *Staphylococcus aureus* cells for 45 min at room temperature. Then 200 $\mu$l anti-$\alpha$-actinin rabbit antiserum was added and the mixture gently shaken for 16 hr at 4°C. Immune complexes were adsorbed with a 10% w/v suspension of glutaraldehyde-fixed *Staphylococcus aureus* [24] for 60 min at room temperature, washed, eluted, and analyzed.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the buffer system and conditions described by Laemmli [25].

### Lipid Analysis

Lipids associated with the cytoskeleton of thrombin-activated platelets or the immunoprecipitated $\alpha$-actinin were extracted by the method of Bligh and Dyer [26]. The chloroform phase containing the extracted lipids was concentrated and the lipid composition was analyzed by two-dimensional high-performance thin-layer chromatography (TLC). The solvents were first dimension—chloroform/methanol/$H_2O$ (65:25:4) and second dimension—n-hexane/chloroform/methanol/diethylether (90:65:25:10). The dried plates were either i) exposed to Kodak XS-5 X-ray film at −80°C to obtain the radioactive profiles, or ii) the separated lipids were scraped from the plates and the amount of radiolabeled lipids was determined by liquid scintillation counting.

### Gel Filtration and Chromatofocusing

Washed cytoskeletons were treated with 100 $\mu$l of 0.6N NaI for 15 min at room temperature. The supernatants obtained after centrifugation (5 min at 10,000g) were used for gel filtration and chromatofocusing experiments.

The dissolved cytoskeletons were applied to Sephadex G-75 columns (1.0 × 10.0 cm) or to columns (1.0 × 3.0 cm) of Polybuffer exchanger PBE 94 (Pharmacia, Uppsala, Sweden) and were eluted with 0.6N NaI or Polybuffer 74 (pH range 6.8–4.0), respectively. Two-milliliter fractions were collected, and aliquots were counted in a Packard liquid scintillation spectrometer.

## RESULTS

### Incorporation of Fatty Acids Into Platelets

Resting human blood platelets were labeled with [$^3$H]-palmitic acid ($C_{16}$, saturated), or [$^3$H]-arachidonic acid ($C_{20}$, tetra-unsaturated) for 2 hr. The total uptake of radiolabeled fatty acids (Table I) and its incorporation into phospholipids were determined. Forty percent of the added palmitic acid compared to 74% of the added arachidonic acid was incorporated into the resting platelets during that time. Labeling with fatty acids resulted in the incorporation of 3% of the label (% of total incorporation into platelets) into phospholipids in the case of palmitic acid and of 90% in the case of arachidonic acid, respectively.

### Association of Lipids With the Cytoskeletons of Resting and Activated Platelets

Lipid binding to the Triton X-100-insoluble cytoskeletons of resting and activated platelets was studied in cells prelabeled with [$^3$H]-fatty acids (Table I). The relative increase of radioactive lipids bound to the cytoskeleton of activated platelets compared with that of resting platelets was 30:1 for labeling with palmitic acid and 2:1 for labeling with arachidonic acid. A low level of binding of radioactive lipids to the cytoskeleton of resting platelets was always observed.

After activation with thrombin for 20 sec, the cytoskeletons were isolated, and after extensive washing were analyzed for their total lipid content. Eighteen percent of the incorporated label was found to be associated with the cytoskeleton of activated platelets when labeled with [$^3$H]-palmitic acid. However, in similar experiments using [$^3$H]-arachidonic acid, only 3% of the label was found to be associated with the cytoskeleton (Table I). Thus the relative binding of lipid components to the cytoskel-

**TABLE I. Incorporation of Lipids Into Resting Platelets and Association of Lipids With Cytoskeletons of Resting and Activated Platelets***

| | | (% of total in platelets) | |
|---|---|---|---|
| Fatty acid | Incorporation into resting platelets (% of total added) | Association with cytoskeletons of resting platelets | Association with cytoskeletons of activated platelets |
| $^3$H-palmitic acid | 40 | 0.6 | 18 |
| $^3$H-arachidonic acid | 74 | 1.5 | 3 |

*The washed platelets were incubated with [$^3$H]-fatty acids in RCD buffer for 2 hr, centrifuged at 10,000g for 4 min, and then washed 3 times with RCD buffer. After solubilization, the radiolabel incorporated into the resting platelets was determined by liquid scintillation counting. Cytoskeletons of resting and thrombin-activated plateles were isolated as Triton X-100-insoluble residues, as described in Materials and Methods. After washing twice with RCD buffer containing 1% Triton X-100 and 5 mM EGTA and once with RCD buffer only, the solubilized cytoskeletons were analyzed for their association with radiolabeled lipids by liquid scintillation counting.

eton of activated, palmitic acid-labeled platelets is six times higher than that to the cytoskeleton of activated platelets labeled with arachidonic acid.

## Diacylglycerol and Fatty Acids Are Associated With the Cytoskeleton of Activated Platelets

High-performance thin-layer chromatography (TLC) was used to analyze the lipids associated with the isolated cytoskeletons in more detail (Table II). Cytoskeletons from activated platelets, prelabeled by either [$^3$H]-palmitic acid or [$^3$H]-arachidonic acid, were isolated, extracted with chloroform-methanol, and lipids were analyzed (see Materials and Methods). Most of the radioactivity attached to the cytoskeleton of palmitic acid-labeled platelets came from palmitic acid (92%), diacylglycerol (4%), and phospholipids (4%). The radioactivity associated with the cytoskeleton of platelets prelabeled with arachidonic acid was found mainly in phospholipids (86%), less in free diacylglycerol (10%), and practically none in free fatty acid (4%). In the latter case, 90% of the total radioactivity in the platelets was incorporated into phospholipids; however, the association of labeled lipids to the cytoskeleton was sixfold less than that of palmitic acid-labeled platelets.

## Diacylglycerol and Palmitic Acid Are Bound to α-Actinin

Gel filtration and chromatofocusing studies were performed to demonstrate a possible tight binding of specific lipid components to certain cytoskeleton-associated proteins. Cytoskeletons from prelabeled ([$^3$H]-palmitic acid or [$^3$H]-arachidonic acid) activated platelets were dissociated in 0.6 N NaI, and the supernatants of dissolved cytoskeletons were then applied to Sephadex G-75 columns. Most of the radioactive label was found to be eluted in the void volume of the column, together with the high molecular weight proteins associated with the cytoskeleton (Fig. 1, fractions 2 and 3). α-Actinin was the major protein in these fractions as demonstrated by analysis of these fractions on SDS-polyacrylamide gels (data not shown). The same qualitative result was obtained using either of the labeled fatty acids. Less than 0.1% of the radioactivity applied stayed trapped in the column after washing with 1% Triton X-100 (Fig. 1).

In similar experiments, dissolved cytoskeletons of activated platelets were applied to columns of ion exchange resin (Polybuffer exchanger PBE 94) and elution was performed with Polybuffer 74. Figure 2 shows the pattern of radioactivity contained in the fractions collected, as well as the pH gradient generated on the column. The radiolabel-containing fractions (Fig. 2, fractions 3 and 4), show pH

**TABLE II. Lipids Associated With the Cytoskeletons of Activated Platelets***

| | Phospholipid (%) | Fatty acid (%) | Diacylglycerol (%) |
|---|---|---|---|
| $^3$H-palmitic acid | 4 | 92 | 4 |
| $^3$H-arachidonic acid | 86 | 4 | 10 |

*The washed platelets were incubated with [$^3$H]-fatty acids in RCD buffer for 2 hr. Cytoskeletons of thrombin-activated platelets were then isolated as Triton X-100-insoluble residues, as described in Materials and Methods. The isolated cytoskeletons were washed twice with RCD buffer containing 1% Triton X-100 and 5 mM EGTA and once with RCD buffer. The lipids associated with the washed cytoskeletons were then extracted by the method of Bligh and Dyer [26] and separated by two-dimensional high-performance TLC. Separated samples were scraped from the plates and radiolabeled lipids were analyzed by liquid scintillation counting.

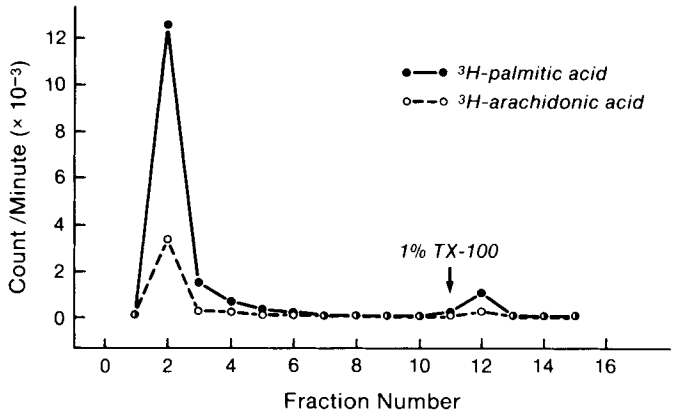


Fig. 1. Gel filtration of dissociated cytoskeletons isolated from thrombin-activated blood platelets. Cytoskeletons were isolated from platelets prelabeled with [$^3$H]-palmitic acid (●) or [$^3$H]-arachidonic acid (○) and were dissociated in 100 μl of 0.6 N NaI. After centrifugation, the supernatants were applied to Sephadex G-75 gel filtration columns (1.0 × 10.0 cm). Elution was performed with 0.6 N NaI and 2-ml fractions were collected. Aliquots were taken and the radioactivity in each fraction was quantitated by liquid scintillation counting. Radiolabeled lipids were eluted in fractions 2 and 3 together with the high molecular weight proteins, such as α-actinin.

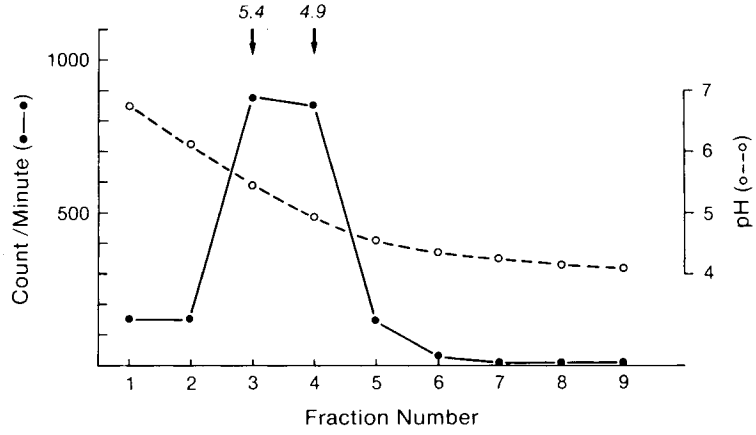


Fig. 2. Chromatofocusing of dissociated cytoskeletons isolated from thrombin-activated blood platelets. Cytoskeletons were isolated from platelets prelabeled with [$^3$H]-palmitic acid and were dissociated in 100 μl of 0.6 N NaI. After centrifugation the supernatant was applied to a column of Polybuffer exchanger PBE 94 (1.0 × 3.0 cm). Elution was performed with Polybuffer 74 (pH range 6.8–4.0) and 2-ml fractions were collected. The collected fractions were analyzed for their content of radioactivity (●) and their pH (○). The radiolabel-containing fractions (fractions 3 and 4) show pH values of 5.4 and 4.9, respectively, corresponding to the pI value range of α-actinin.

values of 5.4 and 4.9, respectively. This corresponds to the pI value range of α-actinin. In addition, analysis of the different fractions by SDS-PAGE demonstrated that α-actinin was mainly contained in fractions 3 and 4 (data not shown).

The association of specific lipids with α-actinin was finally proved by co-immunoprecipitation studies (Fig. 3). α-Actinin was immunoprecipitated from solubilized cytoskeletons of [$^3$H]-palmitic acid-labeled, thrombin-activated cells by anti-α-actinin specific antibodies. Analysis of the immunoprecipitate by SDS-PAGE dem-

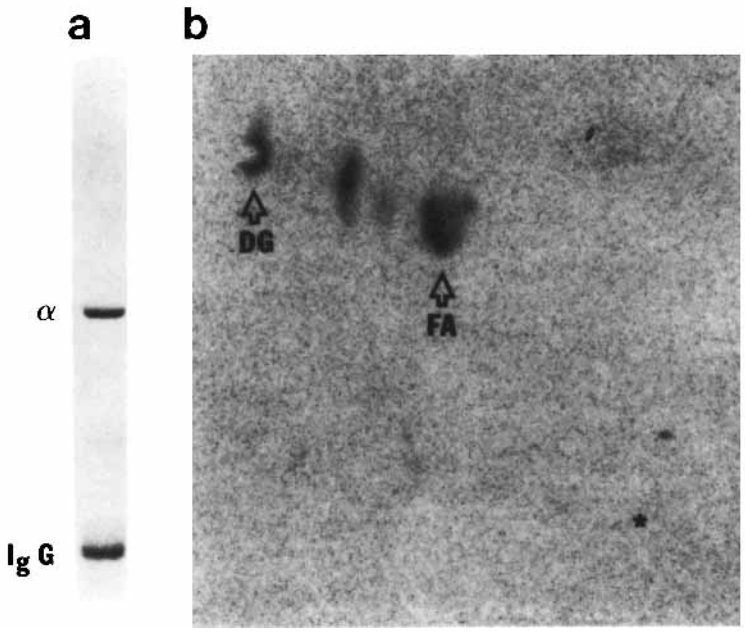


Fig. 3. Diacylglycerol and fatty acids co-immunoprecipitate with α-actinin. α-Actinin was immunoprecipitated from solubilized cytoskeletons of [$^3$H]-palmitic acid-labeled, thrombin-activated platelets, as described in Materials and Methods. The immunoprecipitates were used either for a) protein analysis by SDS-PAGE, or b) analysis of the co-immunoprecipitated lipids by two-dimensional TLC. **a**: SDS-PAGE (0.1% SDS/7.5% polyacrylamide) of the immunoprecipitate. The proteins were visualized by Coomassie blue staining. The positions of α-actinin (α) and immunoglobulin G heavy chain (IgG) are marked. **b**: The lipids associated with the immunoprecipitated α-actinin were extracted and separated by two-dimensional TLC, as described in Materials and Methods. The first dimension (upward) was in chloroform/methanol/$H_2O$ (65:25:4). The second dimension (to the left) was in n-hexane/chloroform/methanol/diethylether (90:65:25:10). The autoradiogram shown was obtained by exposing Kodak XS-5 X-ray film to the dried plate at −80°C for 2 months. DG, diacylglycerol; FA, fatty acids. *Origin of chromatography.

onstrated the precipitation of a single protein having the mobility of α-actinin (Fig. 3a). Further investigation of the immunoprecipitate showed a co-immunoprecipitation of radiolabeled lipids with α-actinin. Moreover, extraction of the immunoprecipitate with organic solvents and analysis of the extracted lipids by two-dimensional TLC demonstrated the specific binding of diacylglycerol and palmitic acid to α-actinin (Fig. 3b). The separated lipids were scraped from the TLC plates and the amount of radiolabeled lipids was determined by liquid scintillation counting. The ratio of diacylglycerol to fatty acid was almost 1:1.

## DISCUSSION

In studies with model membranes a specific interaction of certain lipids with α-actinin has been demonstrated [20]. In monolayer experiments using a modified Langmuir trough, a dramatic increase of the surface viscosity in the α-actinin-lipid layer, concomitant with effective sorting out of lipids in the presence of α-actinin, was described. The formation of these rigid structures, however, was dependent on

the presence of fatty acids and diacylglycerol in the surface layer. One molecule of diacylglycerol and one molecule of fatty acid were found to be associated with one molecule of the α-actinin dimer. Using saturated and mono-unsaturated fatty acids from $C_{14}$ to $C_{22}$, together with saturated or mono-unsaturated mono- and diacylglycerols, gave the same qualitative results. Lipids containing unsaturated fatty acid chains such as arachidonic acid (tetra-unsaturated), did not interact with α-actinin in a similar manner. Other experiments have demonstrated a specific effect of 1,2-diolein and palmitic acid on the formation of supramolecular complexes between α-actinin and actin, indicating an involvement of lipids in the organization of actin-containing microfilaments [21,22].

In this study, lipid binding to the Triton X-100-insoluble cytoskeletal structures of resting and activated platelets was studied. This approach is based on the finding that most platelet proteins, including F-actin, are Triton soluble while organized bundles of filamentous actin and their associated proteins, remain unsoluble under the conditions described. The Triton X-100-insoluble material is termed cytoskeleton [18]. It is reasonable to assume that lipids which are tightly bound (covalent or noncovalent) to the cytoskeleton stay associated with these supramolecular structures during this extraction procedure. Cytoskeletons of activated platelets, prelabeled with [$^3$H]-palmitic acid or [$^3$H]-arachidonic acid, were isolated. The binding of lipid components to the cytoskeleton of activated platelets labeled with palmitic acid was found to be six times higher than for control platelets labeled with arachidonic acid (Table I). Since more arachidonic acid is incorporated into resting platelets (and into phospholipids) than palmitic acid (Table I), the observed increase of relative binding of lipids to the cytoskeleton of activated platelets is even more significant. The reduced association of arachidonic acid labeled lipids with the cytoskeletal elements may be due to a different interaction of these lipids with α-actinin.

Physiological activation of platelets with thrombin leads to a significant attachment of lipids to the cytoskeletal elements. A 2-fold (arachidonic acid) and 30-fold (palmitic acid) increase of radiolabeled lipids associated with the cytoskeleton of activated compared to resting platelets was observed (Table I). A low level of binding of lipids to the cytoskeleton of resting platelets was always observed and may result either from a small population of activated platelets in the preparation, or the binding to the small amount of organized actin filaments reported to be present in resting cells [12,27]. Analysis of the lipids associated with the cytoskeletons of activated platelets demonstrates binding of both diacylglycerol, as well as fatty acids (Table II); in addition, some binding of phospholipids was always observed.

The in vitro studies described above have demonstrated binding of certain fatty acids and diacylglycerol to α-actinin [20]. Together with the observation of i) an increased association of α-actinin with cytoskeletal structures of platelets during receptor-mediated aggregation [12,14,15] and ii) an increased binding of diacylglycerol and fatty acids to the same structures during platelet activation (this study), these results suggest that these lipids bind to the cytoskeleton via α-actinin. Analysis of cytoskeletons of activated platelets by gel filtration studies and chromatofocusing studies indeed indicated a tight binding of lipids to α-actinin. α-Actinin was found in the void volume of Sephadex G-75 columns together with the bulk of radiolabeled lipids (Fig. 1). Chromatofocusing studies showed an association of radiolabeled lipids with a protein having a pI value similar to that of α-actinin (Fig. 2). In addition, analysis of cytoskeletons from [$^3$H]-palmitic acid-labeled, thrombin-activated platelets

by SDS-PAGE showed a single radiolabeled band in the position where α-actinin was migrating [21,22]. The final proof of an association of lipids with α-actinin, however, came from immunoprecipitation studies using anti-α-actinin-specific antibodies (Fig. 3) in which a co-precipitation of diacylglycerol and palmitic acid with α-actinin could be demonstrated (Fig. 3b). The ratio of diacylglycerol to fatty acid was almost 1:1 as was found earlier in monolayer studies [20].

According to these results, a mechanism involving the phosphatidylinositol cycle, the second messenger diacylglycerol, fatty acids, and α-actinin is suggested to be involved in the regulation of cytoskeleton-membrane interactions. Receptor-mediated stimulation of blood platelets may activate phospholipases; the mechanism by which phospholipases become activated is not yet completely known [5]; however, G-proteins may be involved [28,29]. Generation of diacylglycerol from phosphoinositides, and fatty acids from other lipids [1–5], may initiate the formation of stable complexes between the two lipids and a particular fraction of α-actinin [20–22] that is either already associated with the membrane [14] or may now be translocated into the membrane. Activation of the platelets leads at the same time to actin polymerization, reorganization of the cytoskeleton [11,14–16], and a progressive increase in binding of α-actinin to the cytoskeletal structures [12,14,15]. The formation of such α-actinin/lipid complexes can increase the affinity and attachment of membrane-associated α-actinin with the cytoskeleton and therefore could be fundamental to the interaction between the cytoskeleton and the membrane. In the plane of the membrane the interaction of α-actinin/lipid complexes among themselves, as suggested by the rigid monolayer complexes found [20], may also be involved in the initiation of bundle formation [21,22].

Interestingly, phosphatidylinositol 4,5-bisphosphate, another component of the phosphatidylinositol cycle, has been reported to have a specific effect on profilactin, a putative precursor of actin filaments, causing a rapid and efficient dissociation of profilactin with a concomitant polymerization of the actin in appropriate conditions [30]. In addition, the function of the actin-binding protein gelsolin seems to be modulated by phosphatidylinositol 4,5-bisphosphate [31]. Phosphatidylinositol 4,5-bisphosphate strongly inhibits the actin filament-severing properties of gelsolin, inhibits less strongly the nucleating ability of this protein, and restores the potential for filament-severing activity to gelsolin-actin complexes. Thus, receptor-mediated activation of cells may initiate phosphorylation of phosphatidylinositol to phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate which may cause the dissociation of the profilactin and gelsolin-actin complexes and may lead finally to the polymerization of actin. Hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C, the following step in the phosphatidylinositol cycle, generates the second messenger diacylglycerol and may result in its association with α-actinin. Thus, two intermediate lipid molecules generated during the phosphoinositide turnover, phosphatidylinositol 4-5-bisphosphate and diacylglycerol, seem to be important in initiating the formation of actin filaments and their anchoring to membranes.

In summary, the results discussed in this report indicate the possibility that the phosphatidylinositol cycle may be involved in the assembly of actin filaments as well as in the regulation of cytoskeleton-membrane interactions. The second messenger diacylglycerol, fatty acids, and α-actinin may be part of the complex mechanism.

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