Thrombin activation of human platelets dissociates a complex containing gelsolin and actin from phosphatidylinositide-specific phospholipase C γ_1

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We have examined the association of two cytoskeleton proteins, gelsolin and actin, with phosphatidylinositide-specific phospholipase C γ_1 (PLC γ_1) in resting and thrombin-stimulated human platelets. In unstimulated platelets, gelsolin, actin and PLC γ_1 were immunoprecipitated as a complex by a polyclonal antibody to PLC γ_1 . The association of gelsolin and actin was specific for PLC γ_1 because immunoprecipitates of PLCs β_2 , β_3 , γ_2 and δ_1 , which are also expressed in human platelets, did not contain detectable gelsolin or actin. Activation with thrombin resulted in platelet aggregation and the dissociation of gelsolin and actin from PLC γ_1 . Inhibition of thrombin-induced platelet aggregation blocked the dissociation of gelsolin and actin from PLC γ_1 . After

stimulation with thrombin, PLC γ_1 activity in immunoprecipitates was increased 2–3-fold. This elevation in PLC γ_1 activity in response to thrombin activation was not observed when platelet aggregation was blocked. Although PLC γ_1 is tyrosine phosphorylated in response to many agonists, we could not detect, by Western analysis with anti-phosphotyrosine antibodies, tyrosine phosphorylation of PLC γ_1 immunoprecipitated from thrombin-stimulated platelets. These results demonstrate that PLC γ_1 is associated with gelsolin and actin in resting platelets, and that thrombin-induced platelet aggregation results in the dissociation of PLC γ_1 from gelsolin and actin, and the stimulation of PLC γ_1 activity.

INTRODUCTION

A number of platelet agonists including thrombin, thromboxane A $_2$ and collagen have been shown to stimulate phospholipase C-catalysed phosphoinositide hydrolysis (reviewed in [1,2]). Platelets express phosphatidylinositide-specific phospholipase C (PLC) isoenzymes β_2 , β_3 , γ_1 , γ_2 , and δ_1 , and a 140 kDa β_3 isoform [3–6]. Reconstitution studies [4] with isolated membranes and purified platelet PLC isoforms indicate that different platelet agonists activate distinct PLC isoforms. For example, collagen specifically activates PLC γ_2 [7], whereas PLC γ_2 is not stimulated by thrombin (J. J. Baldassare, P. A. Henderson and G. J. Fisher, unpublished work). Studies with platelet membranes and intact platelets have demonstrated that thrombin activation of PLC activity is dependent on coupling through G-protein(s) [8–11]. Several laboratories have also demonstrated that thrombin stimulates tyrosine phosphorylation of several proteins in platelets [12–14], and recent results [15] indicate that thrombin activation of PLC γ_1 might involve a tyrosine-dependent pathway in human platelets.

The cytosolic actin-binding proteins profilin and gelsolin have been shown to affect PLC γ_1 -catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP $_2$) [16,17]. The addition of profilin and/or gelsolin to PIP $_2$ -containing liposomes significantly decreased PIP $_2$ hydrolysis by unphosphorylated PLC γ_1 [16,17]. In contrast, profilin did not effect phosphorylated PLC γ_1 or PLC β activities [16]. Banno et al. [18] have demonstrated that PLC γ_1 is associated with gelsolin and monomeric actin in cytosolic preparations from outdated platelets. In the present study we have characterized the association of gelsolin and actin with PLC γ_1 in soluble extracts from fresh human platelets, and investigated the effect of thrombin activation on the association of gelsolin and

actin with PLC γ_1 . The results demonstrate that, in freshly isolated resting human platelets, gelsolin, actin and PLC γ_1 are associated, and that thrombin-induced platelet aggregation results in the dissociation of this complex and a 3–5-fold increase in PLC γ_1 activity.

EXPERIMENTAL

Materials

Enhanced chemiluminescence kit for immunoblotting and [3 H]PIP $_2$ (3Ci/mmol) were obtained from Amersham Life Sciences (Arlington, IL, U.S.A.). Tyrphostin A47 was purchased from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Thrombin (4580 NIH units/mg), prostaglandin I $_2$, apyrase, RGDS and antibodies to gelsolin and actin were purchased from Sigma Chemical Co. (Saint Louis, MO, U.S.A.). Immobilon-P was purchased from Millipore Corporation. All other compounds were of at least reagent grade.

Platelet preparation

Whole blood was collected from healthy volunteers, and platelet-rich plasma was prepared, within 2 h of collection, by centrifugation at 150 *g* for 15 min at room temperature. Platelets were prepared as described previously [19]. Briefly, 10 ng/ml prostaglandin I $_2$ and 1 unit/ml apyrase were added to platelet-rich plasma, the mixture was layered on a 40% (w/v) BSA cushion and centrifuged at room temperature for 10 min at 1000 *g*. The platelet pellet was resuspended in Tyrode's/Hepes buffer con-

taining 134 mM NaCl, 12 mM NaHCO₃, 3.0 mM KCl, 0.35 mM NaH₂PO₄, 1.0 mM MgCl₂, 5.6 mM dextrose and 10 mM Hepes, pH 7.4. Platelets were then filtered through a Sepharose 4B-CL column equilibrated with Tyrodes/Hepes buffer.

Platelet activation

Gel-filtered platelets were preincubated at 37 °C for 5 min before the addition of thrombin (0.5 unit/ml). Where indicated in the text, platelets were preincubated for 5 min in the presence of 0.5 mM RGDS peptide or 1 mM EGTA before the addition of thrombin. At the indicated times, platelets were pelleted for 20 s in a microcentrifuge, resuspended in 20 mM Tris/HCl (pH 7.4)/10 mM EGTA/1 mM PMSF/1 mM dithiothreitol/10 µg/ml leupeptin/10 µg/ml aprotinin/1 mM sodium vanadate/2 mM sodium pyrophosphate and sonicated for three pulses, each pulse for 10 s. The lysate was centrifuged for 5 min at 2400 g and platelet cytosol was prepared by centrifugation of the supernatant at 100 000 g for 2 h.

Purification of gelsolin-actin complex and gelsolin from human platelets

The gelsolin-actin complex, which was complexed with PLCγ₁, was separated from PLCγ₁ on a hydroxyapatite column with a linear potassium phosphate gradient as described [5]. Free gelsolin was purified from actin by chromatography on DEAE-cellulose as previously described [5]. Actin and gelsolin were quantified by immunoblot analysis with recombinant actin or gelsolin as standards.

SDS/PAGE electrophoresis and immunoblotting

SDS/PAGE and Western blots were performed as previously described [20]. Briefly, samples were resuspended in SDS sample buffer and subjected to electrophoresis in 9% (w/v) polyacrylamide gels [21]. Proteins (50–100 µg) were electroblotted on Immobilon-P; after transfer the Immobilon-P sheets were incubated overnight in wash buffer containing 5% (w/v) dried milk, pH 8.0, as described [22]. The blots were incubated for 1 h at room temperature with primary antibody, washed and then incubated for ½ hour at room temperature with anti-IgG-horseradish peroxidase conjugate. The blots were developed by chemiluminescence detection. Band intensities were quantified by scanning laser densitometry with a Molecular Dynamics Laser Densitometer [20].

Immunoprecipitation

Platelet lysates (1 mg/ml) were precleared with normal rabbit serum to decrease the non-specific binding of lysate proteins to immune complexes. Platelet lysates were incubated with normal rabbit serum (50 µl/ml cytosol) for 1 h at 4 °C. Treated lysates were added to prewashed fixed *Staphylococcus aureus* Cowan I (50 µl/µl of serum); the mixture was incubated at 4 °C for 30 min and then pelleted in a microcentrifuge at 4 °C for 15 min. Precleared lysates (200–400 µg of protein) were incubated with rabbit polyclonal affinity-purified antibodies (2–5 µg/100 µg of protein) to PLCβ₂, β₃ [4], PLCγ₁, γ₂ and δ₁ [4] for 1 h at 4 °C. The quantities of PLC antibodies required for quantitative immunoprecipitation were determined by titration with purified platelet PLC proteins [23]. Immunoreactive proteins were bound to Protein A-Sepharose that had been preincubated with 1 mg/ml BSA for 2 h at 4 °C followed by centrifugation at 1000 g for

5 min. Pellets were washed at least four times with Tris/HCl buffer and immunoblotted as described above.

PLC activity

PIP₂ hydrolysis was performed in 50 µl reaction mixture containing 250 µM [³H]PIP₂ (25 000 c.p.m.), 30 mM NaH₂PO₄, pH 6.9, 0.15% n-octyl β-D-glucopyranoside, 0.05% Triton X-100, 1 µM free Ca²⁺ and 70 mM KCl. The reaction was terminated after 15 min and the inositol phosphates quantified as described previously [9].

Platelet aggregation

Platelets were incubated in the aggregation cuvette and the aggregation reaction initiated by the addition of 0.5 unit/ml thrombin with constant stirring. Macroscopic platelet aggregation was measured turbidimetrically on a Chrono-Log aggregometer (Chrono-Log Corp., Haverton, PA, U.S.A.).

Protein determination

Protein concentrations were determined as described by Schaffner and Weissmann [24].

RESULTS

Association of gelsolin, actin and PLCγ₁ in human platelets

To determine whether PLCγ₁ was associated with gelsolin and actin in fresh human platelets, cytosols from fresh platelets were immunoprecipitated with affinity-purified antibody to PLCγ₁ and the immunoprecipitates were analysed by Western blots probed with antibodies specific for PLCγ₁, gelsolin or actin. Western blot analysis of platelet cytosol before and after immunoprecipitation revealed that only 80–85% of the PLCγ₁ was immunoprecipitated (results not shown). Further addition of antibody did not immunoprecipitate the remaining PLCγ₁, suggesting that approx. 15% of platelet PLCγ₁ exists in a state resistant to immunoprecipitation. As previously observed for outdated human platelets, gelsolin and actin was co-immunoprecipitated with PLCγ₁ in cytosols from fresh human platelets (Figure 1A). Addition of the detergents sodium cholate (1%, w/v) or SDS (0.1%) to platelet cytosols before immunoprecipitation caused the dissociation of gelsolin and actin from PLCγ₁, i.e. only PLC was immunoprecipitated with anti-PLCγ₁ antibodies (Figure 1B). These results indicate that the co-immunoprecipitation of gelsolin, actin and PLCγ₁ is due to a physical association of the three proteins rather than to separate immunoreactivity of each of the three proteins to the PLCγ₁ antibody. To determine whether the association of gelsolin and actin was specific for PLCγ₁, platelet cytosols were immunoprecipitated with specific antibodies for distinct PLC isoenzymes expressed in human platelets [5], PLCβ₂, β₃, γ₂ and δ₁, and the immunoprecipitates were blotted with antibodies to gelsolin and actin. Neither gelsolin or actin (results not shown) were detected in PLCβ₂, β₃ or γ₂ immunoprecipitates. These results demonstrate that gelsolin and actin interact with PLCγ₁, but not with PLCβ₂, β₃, γ₂ or δ₁, in human platelets.

Time course of the dissociation of gelsolin and actin from PLCγ₁ after thrombin activation

Activation of human platelets with thrombin results in numerous biochemical and morphological alterations, including increased hydrolysis of phosphatidylinositides, rearrangement of the cyto-

Figure 1 Association of gelsolin and actin with $PLC\gamma_1$ in resting human platelets

(A) Fresh, gel-filtered platelets were homogenized, and high-speed supernatants were immunoprecipitated with anti- $PLC\gamma_1$ antibody. Immunoprecipitates were analysed by Western blots probed with antibodies to $PLC\gamma_1$ (lane 1), gelsolin (lane 2) or actin (lane 3). (B) Platelet cytosols were immunoprecipitated as described for (A), in the presence of 1% (w/v) sodium cholate. Immunoprecipitates were analysed by Western blots probed with antibodies to $PLC\gamma_1$ (lane 1), gelsolin (lane 2) or actin (lane 3). The positions of molecular mass standards are indicated at the left (in kDa). Thick arrows at the left identify the positions of $PLC\gamma_1$ (top), gelsolin (middle) and actin (bottom). Results are representative of at least three experiments with similar results.

Figure 2 Time course of thrombin-induced dissociation of gelsolin and actin from $PLC\gamma_1$

Human platelets were treated with thrombin (0.5 unit/ml) at 37 °C with constant stirring. At the indicated times high-speed supernatants were prepared and $PLC\gamma_1$ was immunoprecipitated with anti- $PLC\gamma_1$ antibodies. The immunoprecipitates were analysed by Western blot with antibodies directed against $PLC\gamma_1$, gelsolin and actin as described in the Experimental section. Platelet aggregation, which is shown at the bottom of the figure, was quantified at the indicated times as described in the Experimental section, and is presented as a percentage of the maximal aggregation (determined after 2 min with 0.5 unit/ml thrombin). The results are representative of at least five independent experiments with variations of less than 20%.

skeleton, and aggregation. We therefore investigated whether gelsolin, actin and $PLC\gamma_1$ remained associated after the activation of human platelets by thrombin. Platelets were treated with thrombin (0.5 unit/ml) in the presence of 1 mM Ca^{2+} for up to 5 min with stirring, after which the association of gelsolin and actin with $PLC\gamma_1$ was assessed in anti- $PLC\gamma_1$ immunoprecipitates, as described above. Activation by thrombin resulted in a time-dependent dissociation of the complex (Figure 2).

Figure 3 Thrombin-induced platelet aggregation and dissociation of gelsolin and actin from $PLC\gamma_1$

Human platelets (10^9 /ml) were incubated without (lane 1) or with (lane 2) thrombin (0.5 unit/ml) for 5 min at 37 °C with constant stirring. In lanes 3 and 4, human platelets were incubated with thrombin (0.5 unit/ml) without stirring, or preincubated with 0.5 mM RGDS peptide for 5 min at 37 °C before the addition of thrombin; five minutes after thrombin addition, high-speed supernatants were prepared and immunoprecipitated with anti- $PLC\gamma_1$ antibodies. Immunoprecipitates were analysed by Western blot probed with antibodies to $PLC\gamma_1$, gelsolin or actin (indicated at the right), as described in the Experimental section. Platelet aggregation was measured after 2 min at 37 °C and is expressed as a percentage of the maximal aggregation (observed with 0.5 unit/ml thrombin). Results are representative of four experiments, with variations between experiments of approximately 17%.

Maximal dissociation occurred approx. 1–2 min after thrombin addition (Figure 2). In contrast with the dissociation of the complex, platelet aggregation was complete 30 s after thrombin addition.

Effect of platelet aggregation on the association of gelsolin and actin with $PLC\gamma_1$

We next examined the importance of platelet aggregation on the dissociation of the complex. In the presence of 1 mM Ca^{2+} , 0.5 unit/ml thrombin and stirring, platelet aggregation was complete at approximately 30 s (Figure 2), demonstrating that platelet aggregation preceded the dissociation of the complex. To determine whether the dissociation of gelsolin and actin from $PLC\gamma_1$ was dependent on aggregation, two approaches were taken. First, platelets were incubated with 0.5 unit/ml thrombin/1 mM Ca^{2+} in the absence of stirring. In the second approach platelets were incubated with either the RGDS peptide (0.5 mM) or EGTA (1 mM) before the addition of thrombin, to block aggregation. Not stirring the platelets and pretreatment with either RGDS or EGTA (results not shown) inhibited aggregation by more than 80% (Figure 3), and completely blocked the dissociation of gelsolin and actin from $PLC\gamma_1$ (Figure 3). These results indicate that platelet aggregation is required for the thrombin-induced dissociation of gelsolin and actin from $PLC\gamma_1$.

Increased $PLC\gamma_1$ activity is associated with dissociation from gelsolin and actin

We next examined whether $PLC\gamma_1$ activity in anti- $PLC\gamma_1$ immunoprecipitates was elevated after thrombin stimulation. Platelets were incubated with thrombin (0.5 unit/ml), with or without stirring for 3 min. $PLC\gamma_1$ was then immunoprecipitated as described above and $PLC\gamma_1$ activity in the immunoprecipitates was determined with PIP_2 as substrate. After thrombin treatment, $PLC\gamma_1$ activity was elevated 2–3-fold only when the platelets

Table 1 Thrombin induces PLC γ_1 activity in human platelets

Human platelets were incubated without or with constant stirring at 37 °C with or without 0.5 unit/ml thrombin, alone or in the presence of RGDS peptide. After 5 min the platelets were lysed and PLC γ_1 was immunoprecipitated as described in the Experimental section. The PLC γ_1 immunoprecipitates were resuspended in PIP₂ hydrolysis buffer and PIP₂ hydrolysis was determined as described in the Experimental section. Results are presented as means $\pm$ S.D. for three separate experiments. Abbreviation: IP₃, inositol 1,4,5-trisphosphate.

Additions	[³ H]IP ₃ release (pmol/min)
None	2.83 $\pm$ 0.73
Thrombin (stirring)	8.61 $\pm$ 1.15
Thrombin (no stirring)	3.90 $\pm$ 1.17
Thrombin (RGDS)	3.31 $\pm$ 0.94

Table 2 Effect of gelsolin/actin complex on PLC γ_1 activity

Human platelets (10⁹/ml) were incubated without or with thrombin (0.5 unit/ml) with constant stirring at 37 °C. After 5 min the platelets were lysed and the immunoprecipitates were resuspended in PIP₂ hydrolysis buffer. To determine the effect of gelsolin and gelsolin plus actin on PLC γ_1 -dependent PIP₂ hydrolysis, PLC γ_1 was incubated for 20 min at 30 °C with either gelsolin plus actin or gelsolin alone in molar ratios of 1:1:1 or 1:1:1 respectively. PIP₂ hydrolysis was quantified as described in the Experimental section. PLC γ_1 activity in immunoprecipitates from control platelets (resting platelets) was 2.3 $\pm$ 0.33 (pmol/min). Results are presented as means $\pm$ S.D. for three separate experiments. Abbreviation: IP₃, inositol 1,4,5-trisphosphate.

Conditions	[³ H]IP ₃ release (% of control)
Control	100 $\pm$ 15
Control + (gelsolin/actin)	96 $\pm$ 19
Control + gelsolin	38 $\pm$ 14
Thrombin	321 $\pm$ 21
Thrombin + (gelsolin/actin)	337 $\pm$ 13
Thrombin + gelsolin	36 $\pm$ 17

were stirred (Table 1). Western blots of the immunoprecipitates demonstrated that the observed increase in PLC γ_1 activity after thrombin treatment was not due to increased amounts of immunoprecipitated PLC γ_1 protein (Figure 2). The elevated activity was not due to the absence of the complex containing gelsolin and actin in the immunoprecipitates themselves, because addition of the complex (gelsolin-actin) to the immunoprecipitates had no effect on PLC γ_1 activity from either resting or thrombin-stimulated platelets (Table 2). In contrast, the addition of gelsolin alone significantly inhibited the PLC γ_1 activity of resting or thrombin-stimulated platelets (Table 2).

Thrombin does not induce detectable tyrosine phosphorylation of PLC γ_1

Agonists such as PDGF and EGF, which bind to cell surface receptors with intrinsic tyrosine kinase activity, induce tyrosine phosphorylation of PLC γ_1 [25,26]. Treatment with EGF also resulted in a significant enhancement in PLC γ_1 , suggesting that tyrosine phosphorylation of PLC γ_1 results in increased activity [25]. Several studies have shown that in human platelets thrombin treatment results in the enhanced tyrosine phosphorylation of a number of proteins [12,13]. We therefore examined whether thrombin-induced stimulation of PLC γ_1 was associated with increased tyrosine phosphorylation of PLC γ_1 . PLC γ_1 was immunoprecipitated from control and thrombin-stimulated platelet cytosols, and the immunoprecipitates were analysed by Western blots probed with anti-phosphotyrosine and anti-PLC γ_1 antibodies. No phosphotyrosine could be detected in the PLC γ_1

Figure 4 Thrombin does not induce detectable tyrosine phosphorylation of PLC γ_1 in human platelets

Human platelets (10⁹/ml) were incubated with constant stirring at 37 °C with no addition (lane 1), with 0.5 unit/ml thrombin (lane 2) or with 400 μ M peroxyvanadate (lane 3). After 5–10 min the platelets were lysed and PLC γ_1 was immunoprecipitated as described in the Experimental section. The immunoprecipitates were resuspended in Laemmli buffer and subjected to Western blot analysis with anti-PLC γ_1 (A) or anti-phosphotyrosine (B) antibodies. These results are representative of five experiments with similar results.

Figure 5 Effect of tyrosine phosphorylation inhibitor tyrphostin A47 on thrombin-induced dissociation of gelsolin and actin from PLC γ_1

Human platelets (10⁹/ml) were preincubated for 5 min at 37 °C with no addition or with 20 μ M tyrphostin before the addition of 0.5 unit/ml thrombin; 5–10 min after the thrombin treatment the platelets were lysed and PLC γ_1 was immunoprecipitated as described in the Experimental section. The immunoprecipitates were resuspended in Laemmli buffer and subjected to Western blot analysis with antibodies directed against PLC γ_1 , gelsolin and actin. Lane 1, no additions; lane 2, thrombin; lane 3, thrombin plus tyrphostin. These results are representative of three experiments with similar results.

bands (Figure 4B, lanes 1 and 2). As positive control for PLC γ_1 tyrosine phosphorylation, platelets were treated with the tyrosine phosphatase inhibitor peroxyvanadate (400 μ M), which has been shown to induce tyrosine phosphorylation of PLC γ_1 [27]. Cytosolic PLC γ_1 was immunoprecipitated; the immunoprecipitates were immunoblotted with anti-phosphotyrosine antibodies. As expected, peroxyvanadate treatment resulted in detectable tyrosine phosphorylation of PLC γ_1 (Figure 4B, lane 3). Guinebault et al. [15] have also reported being unable to demonstrate tyrosine phosphorylation of PLC γ_1 in thrombin-stimulated platelets. These results make it unlikely that increased PLC γ_1 activity observed in thrombin-treated platelets (Table 1) is due to tyrosine phosphorylation of PLC γ_1 .

Effect of the tyrosine phosphorylation inhibitor tyrphostin A47 on thrombin-induced dissociation of gelsolin and actin from PLC γ_1

We next investigated whether gelsolin, actin and PLC γ_1 remained associated after thrombin treatment, if thrombin-induced protein

tyrosine phosphorylation was blocked. Preincubation of human platelets with the tyrosine kinase inhibitor tyrphostin A47 blocked thrombin-induced tyrosine phosphorylation of several proteins, including pp125^{Fak}, as judged by Western blot analysis with anti-phosphotyrosine antibodies (results not shown). PLC γ_1 immunoprecipitates of thrombin-treated platelets preincubated with tyrphostin A47 contained gelsolin and actin (Figure 5). These results demonstrate that tyrosine phosphorylation is essential for the thrombin-induced dissociation of gelsolin and actin from PLC γ_1 .

DISCUSSION

We have demonstrated that PLC γ_1 in intact resting platelets is complexed with gelsolin and actin and that this complex can be dissociated by detergents. These results are consistent with those of Banno et al. [18], who previously demonstrated that a complex containing gelsolin and actin co-purified with PLC γ_1 .

Thrombin-induced activation of human platelets resulted in a loss of gelsolin and actin from the anti-PLC γ_1 immunoprecipitates. In contrast with the effect of thrombin alone, not stirring the platelets or preincubation of platelets with RGDS peptide or EGTA markedly inhibited the thrombin-induced dissociation of gelsolin and actin from PLC γ_1 . Addition of the tyrosine phosphorylation inhibitor tyrphostin A47 markedly decreased the ability of thrombin to dissociate the complex (Figure 5). These conditions also significantly blocked platelet aggregation, suggesting that platelet aggregation is required for complex dissociation.

It has been reported that thrombin activation of human platelets induces the tyrosine phosphorylation of several proteins [12–14] including PLC γ_1 [15]. In contrast with these results, immunoblots of anti-PLC γ_1 immunoprecipitates did not detect a protein at 145 kDa when immunoblotted with an antibody directed against anti-phosphotyrosines. This was not due to an inability of the anti-phosphotyrosine antibody to recognize the phosphotyrosine form of PLC γ_1 because the immunoblot analysis clearly identified the tyrosine-phosphorylated form of PLC γ_1 after treatment of platelets with peroxyvanadate (Figure 4). Therefore under our conditions we do not have any evidence that the treatment of human platelets with thrombin results in tyrosine phosphorylation of PLC γ_1 .

At present the role of the association of gelsolin and actin with PLC γ_1 is unknown. Our results demonstrate that the thrombin-induced dissociation of PLC γ_1 is associated with increased activity. However, whereas thrombin induces phosphoinositide hydrolysis within seconds and this hydrolysis is independent of aggregation, the dissociation of gelsolin and actin from PLC γ_1 with the resulting activation of PLC γ_1 occurs within minutes and requires aggregation. Therefore the activation of PLC γ_1 occurs

relatively late and is presumably not involved in the immediate responses of platelets to thrombin. In conclusion, our results clearly demonstrate that in resting platelets a complex containing gelsolin and actin is associated with PLC γ_1 and that the activation of platelets with thrombin induces the dissociation of this complex.

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