pp60$^{c\text{-}src}$ associates with the SH2-containing inositol-5-phosphatase SHIP1 and is involved in its tyrosine phosphorylation downstream of αIIbβ3 integrin in human platelets

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SH2-containing inositol-5-phosphatase 1 (SHIP1) was originally identified as a 145 kDa protein that became tyrosine-phosphorylated in response to multiple cytokines. It is now well established that SHIP1 is specifically expressed in haemopoietic cells and is important as a negative regulator of signalling. We found recently that SHIP1 was present in human blood platelets as an Ins(1,3,4,5)P_4-phosphatase and a PtdIns(3,4,5)P_3-5-phosphatase that became tyrosine-phosphorylated and was relocated to the cytoskeleton in an integrin-dependent manner. Here we report biochemical and pharmacological evidence that the tyrosine kinase pp60$^{c\text{-}src}$ is constitutively associated with SHIP1 and is involved in its tyrosine phosphorylation downstream of integrin engagement in thrombin-activated human platelets. The use of cytochalasin D allowed us to demonstrate that the actin cytoskeleton reorganization induced on thrombin stimulation was not required for its integrin-mediated phosphorylation. Moreover, the integrin-dependent relocation of SHIP1 to the cytoskeleton did not require its tyrosine phosphorylation. These results suggest that SHIP1 is first recruited to the integrin-linked signalling complexes and then becomes tyrosine-phosphorylated through a Src-kinase-dependent mechanism but independently of the actin cytoskeleton reorganization.

Key words: cytoskeleton, inositol-5-phosphatase, non-receptor tyrosine kinase, platelet signal transduction.

INTRODUCTION

The tyrosine phosphorylation of specific regulatory proteins is intimately involved in controlling cell growth and differentiation as well as blood platelet responses [1]. Distinct waves of tyrosine phosphorylations occur in thrombin-stimulated platelets [2]. Fibrinogen binding to the integrin αIIbβ3 tightly controls the last wave of tyrosine phosphorylation during platelet aggregation [3,4]. After ligand engagement, the cytoplasmic domain of integrins associates with functional multiprotein complexes that become closely bound to the actin cytoskeleton on aggregation [5]. The formation of this protein network at focal adhesion sites is responsible for the generation of specific integrin-induced events and for the irreversible aggregation of platelets [5,6]. Among the signalling proteins belonging to this network, we have recently identified the haemopoiesis-specific 145 kDa SH2-domain-containing inositol-5-phosphatase 1 (SHIP1). In recent years, SHIP1 has been extensively studied in immunocompetent cells, where it has a critical role in the negative regulation mediated by FcγRIIB receptor [7]. SHIP1 was first identified as a tyrosine-phosphoprotein associated with Shc in response to several cytokines, including erythropoietin, Steel factor, interleukin 3, interleukin 2, granulocyte/macrophage colony-stimulating factor or macrophage colony-stimulating factor [8,9]. Cross-linking of the B-cell antigen receptor or T-cell activation also induces SHIP1 phosphorylation [10].

Human blood platelet was the first model that permitted the demonstration of the tyrosine phosphorylation and relocation of SHIP1 to the cytoskeleton under the control of an adhesive receptor, the integrin αIIbβ3 [11]. Recently, another example of SHIP1 regulation via an adhesive protein, platelet endothelial cell adhesion molecule 1 (PECAM1), in myelomonocyte (THP1) cells treated by pervanadate has been described [12]. In this case, the immunoreceptor tyrosine-based activation motif (ITAM)-like domain of PECAM1, once phosphorylated, is able to associate with SHIP1. It is interesting to note that PECAM-1 is also present in platelets as a functional protein [13] that might be involved in the regulation of integrin activation.

The study of SHIP1-deficient mice [14] or the microinjection of SHIP1 in *Xenopus* oocytes [15] revealed that this inositol-5-phosphatase performs a large part of its biological roles by regulating events dependent on phosphoinositide 3-kinase. Indeed, this phosphatase has been shown to hydrolyse the 5-position from Ins(1,3,4,5)P_4 and PtdIns(3,4,5)P_3 both *in vitro* and *in vivo*. In human blood platelets, PtdIns(3,4,5)P_3 is produced rapidly and transiently, whereas PtdIns(3,4)P_2 is synthesized more slowly and accumulates, to a large extent, in an aggregation- and integrin-dependent manner [16,17]. There is now compelling evidence that this particular lipid is involved in the irreversible phase of platelet aggregation induced by physiological concentrations of agonist [18,19]. We observed a striking correlation between the kinetics of PtdIns(3,4)P_2 accumulation and the

Abbreviations used: FAK, focal adhesion kinase; ITAM, immunoreceptor tyrosine-based activation motif; PECAM, platelet endothelial cell adhesion molecule; PP-1, pyrazolopyrimidine-type inhibitor 1; SHIP1, SH2-domain-containing inositol-5-phosphatase 1.

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tyrosine phosphorylation and relocation of SHIP1 on stimulation by thrombin. Although several metabolic pathways might contribute to the accumulation of PtdIns(3,4)P_2 [8], SHIP1 might be involved in its formation by the dephosphorylation of PtdIns(3,4,5)P_3. Thus SHIP1 might down-regulate PtdIns(3,4,5)P_3-dependent events, as shown in immunocompetent cells [20,21], and initiate PtdIns(3,4)P_2-mediated mechanisms. A second member of this inositol-5-phosphatase family, SHIP2, has recently been cloned, seems to be ubiquitously expressed and might contribute to the production of PtdIns(3,4)P_2 [22].

The role of the tyrosine phosphorylation of SHIP1, observed in various haemopoietic cells after various stimuli, is still poorly understood. The physiological tyrosine phosphorylation that occurs in different cells does not seem to modulate the intrinsic 5-phosphatase activity towards Ins(1,3,4,5)P_4 or PtdIns(3,4,5)P_3 [11–22]. However, the phosphorylation of SHIP1 by overexpressed Lck leads to decreased activity [23]. Nevertheless, the tyrosine phosphorylation of the two Asn-Pro-Xaa-Tyr (NPXY) motifs of SHIP1 is obviously involved in the regulation of the interaction with partners, including Shc in interleukin-3-stimulated myeloid cells [24] and activated T-lymphocytes [25]. These specific interactions might have a role in localizing SHIP1 where its activity is required. However, SHIP1 might also have a role as a docking protein through its functional domains, allowing the formation of a suitable multiprotein complex [26].

The present study was performed to characterize the tyrosine kinase activity responsible for the integrin-dependent phosphorylation of SHIP1 in human platelets stimulated by thrombin.

MATERIALS AND METHODS

Materials

The anti-peptide antiserum to murine SHIP1 was raised in rabbit and used as described previously [11]. The mouse anti-phosphotyrosine antibody 4G10 and the Src-family kinases antibody (used in immunoprecipitation) were purchased from Euromedex (Strasbourg, France) and Tebu (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), respectively. The monoclonal anti-pp60^{src} antibody used in Western blotting was obtained from Oncogene Research Products (France Biochem). All other reagents were purchased from Sigma unless indicated otherwise.

Preparation and activation of platelets

Platelet concentrates were obtained from the local blood bank (Centre Régional de Transfusion Sanguine, Toulouse, France) and washed platelets were prepared as described previously [27]. Platelets were used at 2×10^9 cells/ml and stimulated with 1 i.u./ml of thrombin at 37 °C and under gentle shaking (150 strokes/min). When indicated, the platelets were preincubated, at 37 °C, for 1 min with 500 μM Arg-Gly-Asp-Ser (RGDS) peptide, for 2 min with 10 μM cytochalasin D or for 15 min with 10 μM pyrazolopyrimidine-type inhibitor 1 (PP-1) (BioMol). Platelet aggregation was measured at 37 °C with a turbidimetric method by using a dual-channel Payton aggregometer (Payton Associates, Scarborough, Ontario, Canada) at 900 rev/min (10^9 platelets/ml).

Immunoprecipitations

Reactions (10^9 cells in 0.5 ml) were stopped by the addition of 1 vol. of ice-cold lysis buffer containing 100 mM Tris/HCl (pH 7.5)/200 mM NaCl/10 mM EDTA/2 % (v/v) Brij (9 lauryl ether, $C_{12}E_9$)/4 mM Na_3VO_4/2 mM PMSF containing 20 μg/ml aprotinin and 20 μg/ml leupeptin. When immunoprecipitates were used for the kinase assay *in vitro*, platelets were lysed in the same buffer supplemented with 20 mM EGTA and 40 μg/ml calpeptin. After gentle shaking for 20 min at 4 °C, insoluble material was removed by centrifugation (12000 ***g*** for 10 min at 4 °C), and the supernatant was precleared for 30 min at 4 °C with Protein A–Sepharose CL4B. The precleared suspensions were then incubated for 1 h at 4 °C with the anti-SHIP1 or the anti-Src-family kinases antibodies and the immune complexes were precipitated by the addition of 50 μl of 10 % (w/v) Protein A–Sepharose CL4B for 1 h. After centrifugation (6000 ***g*** for 5 min at 4 °C), the immunoprecipitates were washed four times with a buffer containing 50 mM Tris/HCl, pH 7.4, 100 mM NaCl, 0.1 % (v/v) Brij, 2 mM Na_3VO_4, 5 μg/ml aprotinin and 5 μg/ml leupeptin and finally boiled in SDS/PAGE sample buffer. The immunoprecipitates used for kinase assay *in vitro* were washed twice with a buffer containing 50 mM Tris/HCl, pH 8, and 500 mM LiCl, and once with the kinase assay buffer containing 50 mM Tris/HCl, pH 7.4, 10 mM $MnCl_2$ and 2.5 mM $MgCl_2$.

Kinase assay *in vitro*

Anti-SHIP1 immunoprecipitates were resuspended in 40 μl of kinase assay buffer and reactions were started by the addition of 10 μM ATP and 10 μCi of [γ-^{32}P]ATP (3000 Ci/mmol; NEN Life Science Products, Le Blanc Mesnil, France). When indicated, 10 μM PP-1 inhibitor was added. Reactions were performed at 25 °C for 20 min under shaking and stopped by the addition of electrophoresis sample buffer. The proteins were then separated by SDS/PAGE [7.5 % (w/v) gel] and the radioactivity incorporated in the proteins was detected and quantified by a PhosphorImager 445 SI (Molecular Dynamics).

Isolation of platelet cytoskeletons

Reactions were stopped and the cytoskeleton was immediately isolated by adding 1 vol. of ice-cold 2 × CSK buffer [100 mM Tris/HCl (pH 7.4)/20 mM EGTA/2 mM Na_3VO_4/4 μg/ml aprotinin/4 μg/ml leupeptin/2 mM PMSF/2 % (v/v) Triton X-100] to control suspensions or those containing activated platelets. After 10 min at 4 °C, the cytoskeleton was recovered by centrifugation (12000 ***g*** for 10 min at 4 °C), washed once in 1 × CSK buffer containing 0.5 % (v/v) Triton X-100 and once with the same buffer without Triton X-100, then resuspended in the electrophoresis sample buffer.

Gel electrophoresis and immunoblotting

Proteins were separated by SDS/PAGE [7.5 % (w/v) gel], transferred to nitrocellulose (Gelman Sciences) and immunoblotted with the use of standard procedures. Immunoblots were developed with the enhanced chemiluminescence system (ECL®; Amersham, Little Chalfont, Bucks., U.K.) and densitometric analysis was performed with a Gel Doc 1000 system (Bio-Rad).

RESULTS

Functional association of SHIP1 with pp60$^{c\text{-}src}$ in human platelets

When anti-SHIP1 immunoprecipitates from resting platelets were probed with anti-phosphotyrosine antibodies, SHIP1 seemed to be tyrosine-phosphorylated and associated with a phosphotyrosyl protein with a molecular mass of approx. 64 kDa

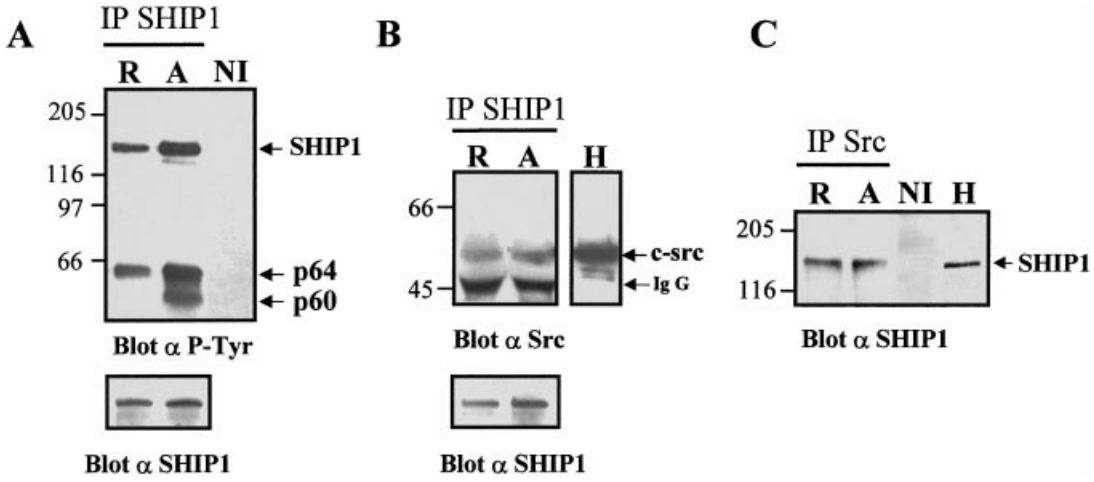

Figure 1 SHIP1 interacts with pp60$^{c\text{-}src}$ in human platelets

Anti-SHIP1 immunoprecipitates (IP SHIP1) from resting platelets (R) or from platelets after activation by 1 i.u./ml thrombin for 3 min (A) were subjected to immunoblotting with 4G10 anti-phosphotyrosine antibody (α P-Tyr) (**A**) or with anti-pp60^{src} monoclonal antibody (α Src) (**B**). The nitrocellulose membranes were stripped and reprobed with anti-SHIP1 antibody (α SHIP1) (lower panels) as a loading control. (**C**) Immunoprecipitates with anti-(Src kinases) (IP Src) from resting platelets or from platelets after activation by 1 i.u./ml thrombin for 3 min were subjected to immunoblotting with anti-SHIP1 antibody. Results are representative of five (**A**), two (**B**) and three (**C**) experiments. NI, immunoprecipitations performed with non-immune serum as a control; H, total homogenate, as a positive control. The positions of molecular mass markers are indicated (in kDa) at the left of each panel.

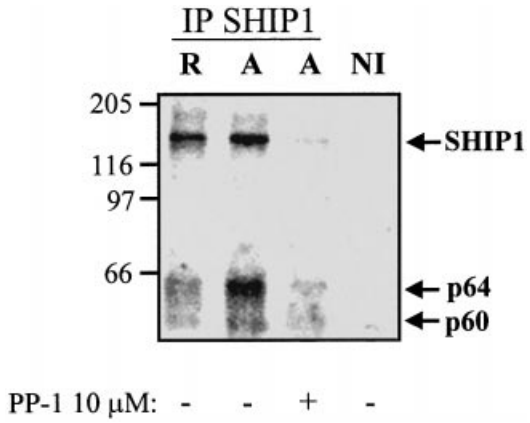

Figure 2 Protein kinase activity, sensitive to the Src-kinase inhibitor PP-1, immunoprecipitates with SHIP1

Anti-SHIP1 immunoprecipitates from resting platelets (R) or from platelets after activation by 1 i.u./ml thrombin for 3 min (A) were subjected to a kinase assay *in vitro* in the presence or the absence of 10 μM PP-1 as indicated in the Materials and methods section. The ^{32}P-labelled proteins were then detected with a PhosphorImager. As a control, a kinase assay performed *in vitro* in an immunoprecipitate done with non-immune serum (NI) gave no radioactive signal. Results are representative of three independent experiments. The positions of molecular mass markers are indicated (in kDa) at the left.

(Figure 1A). The tyrosine phosphorylation of both SHIP1 and its associated protein clearly increased after stimulation by thrombin. In addition, a third phosphotyrosyl protein of molecular mass approx. 60 kDa was detected after stimulation (Figure 1A). The molecular mass of this protein suggested that it could be a tyrosine kinase of the Src family. To check this hypothesis, we performed anti-SHIP1 immunoprecipitations from resting or thrombin-activated platelets and probed them with a specific anti-pp60^{src} antibody. Figure 1(B) shows that pp60$^{c\text{-}src}$ was associated with SHIP1 in resting as well as activated platelets. To confirm this observation, immunoprecipitates with anti-(Src kinases) were probed with the anti-SHIP1-specific antibody. Although pp60$^{c\text{-}src}$, Yes and Fyn were supposed to be recognized by this antibody, pp60$^{c\text{-}src}$ was the major kinase immunoprecipitated (results not shown), in agreement with the fact that it is the major Src kinase in platelets. Figure 1(C) confirms the constitutive association of SHIP1 with pp60$^{c\text{-}src}$ because comparable amounts of SHIP1 were co-immunoprecipitated with the anti-(Src kinase) antibody from resting or thrombin-activated platelets.

When kinase assays were performed *in vitro* in anti-SHIP1 immunoprecipitates obtained from thrombin-activated platelets, three radiolabelled proteins were observed. One phosphoprotein (p145) perfectly matched SHIP1, a second abundant phosphoprotein (p64) matched the unidentified phosphotyrosyl protein associated with SHIP1 and a third phosphoprotein probably corresponded to pp60$^{c\text{-}src}$ (Figure 2). The increase in the phosphorylation of SHIP1, p64 and p60 after stimulation by thrombin was strongly impaired by addition of PP-1, the recently developed inhibitor of Src kinases [28,29]. These results indicate that a protein kinase activity sensitive to PP-1 was co-immunoprecipitated with SHIP1 and was able to phosphorylate SHIP1 and its associated proteins *in vitro*.

By immunoblotting experiments with antibodies specifically directed against Syk and the focal adhesion kinase (FAK) we were able to exclude the association of SHIP1 with these two tyrosine kinases in either resting or thrombin-stimulated platelets (results not shown). Moreover, when SHIP1 was immunoprecipitated from ^{32}P-labelled platelets, we observed that it was weakly serine/threonine phosphorylated under resting conditions and, in contrast with its tyrosine phosphorylation, this phosphorylation did not change after stimulation by thrombin (results not shown). These observations underline the specificity of the interaction between SHIP1 and Src.

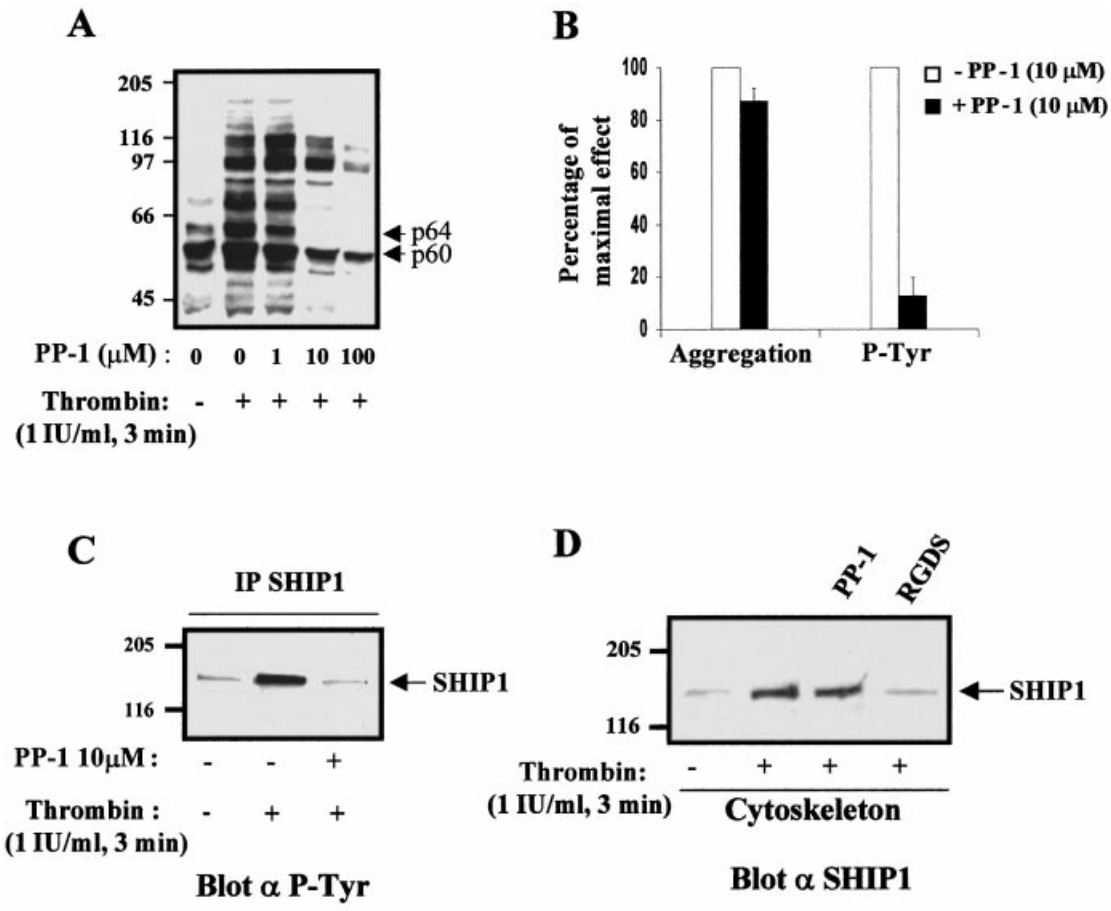

Figure 3 Pharmacological evidence for the involvement of a Src-kinase in the integrin-dependent tyrosine phosphorylation of SHIP1

The effect of increasing doses of the Src kinase inhibitor PP-1 on protein tyrosine phosphorylation induced by 1 i.u./ml thrombin for 3 min was evaluated by immunoblotting platelet whole proteins with the 4G10 anti-phosphotyrosine antibody (α P-Tyr) (**A**). The effect of 10 μM PP-1 (black bars) on 1 i.u./ml thrombin-induced platelet aggregation and tyrosine phosphorylation of SHIP1 was evaluated as described in the Materials and methods section (**B**, **C**). The effect of both PP-1 and RGDS peptide on the relocation of SHIP1 to the cytoskeleton was measured by immunoblotting cytoskeletal proteins extracted from resting platelets or from platelets stimulated by 1 i.u./ml thrombin for 3 min, preincubated or not with 10 μM PP-1 or 200 μg/ml RGDS. Results are representative of two (**A**, **D**) and five (**B**, **C**) experiments. α SHIP1, anti-SHIP1. The positions of molecular mass markers are indicated (in kDa) at the left of (**A**, **C** and **D**).

PP-1, an inhibitor of Src kinases, blocks the integrin-dependent tyrosine phosphorylation of SHIP1 but not its translocation to the cytoskeleton

The role of Src kinases in the integrin-dependent phosphorylation of SHIP1 in human platelets stimulated with thrombin was investigated further by using the PP-1 inhibitor. Increasing amounts of PP-1 were used to determine the concentration necessary for a significant effect on the global tyrosine phosphorylations (Figure 3A) without inhibiting platelet aggregation. A dose of 10 μM PP-1 was found to be effective in inhibiting a number of tyrosine phosphorylations induced by thrombin and did not significantly affect integrin engagement and platelet aggregation (Figure 3B). Under these conditions, the tyrosine phosphorylation of SHIP1 was fully abolished (Figures 3B and 3C). We have shown previously that SHIP1 was relocated to the actin cytoskeleton in an integrin-dependent manner after stimulation by thrombin [11]. Here we show that, interestingly, the tyrosine phosphorylation of SHIP1 was not necessary for its translocation to the cytoskeleton. Indeed, treatment with 10 μM PP-1 fully inhibited the integrin-dependent tyrosine phosphorylation of SHIP1 without affecting its integrin-dependent translocation to the Triton X-100 insoluble material (Figures 3C and 3D). Under similar conditions the RGDS peptide, which we used as a competitor for fibrinogen binding to the integrin αIIbβ3, inhibited the tyrosine phosphorylation of SHIP1.

Actin cytoskeleton reorganization is not required for the integrin-dependent tyrosine phosphorylation of SHIP1

As shown in Figure 4(A), pretreatment with 10 μM cytochalasin D for 2 min almost totally prevented the assembly of actin and the interaction of several actin-binding proteins, including ABP, myosin and α-actinin, with the cytoskeleton of thrombin-stimulated platelets. Interestingly, this inhibition of actin cytoskeleton reorganization by cytochalasin D did not affect the platelet aggregation induced by 1 i.u./ml thrombin (results not shown). Under these conditions, the association of SHIP1 with the Triton X-100-insoluble material was totally inhibited, whereas its tyrosine phosphorylation was not affected (Figures 4B and 4C). In contrast, the inhibition of integrin engagement by RGDS peptide completely inhibited these two events without affecting the translocation of the major actin-binding proteins to the cyto-

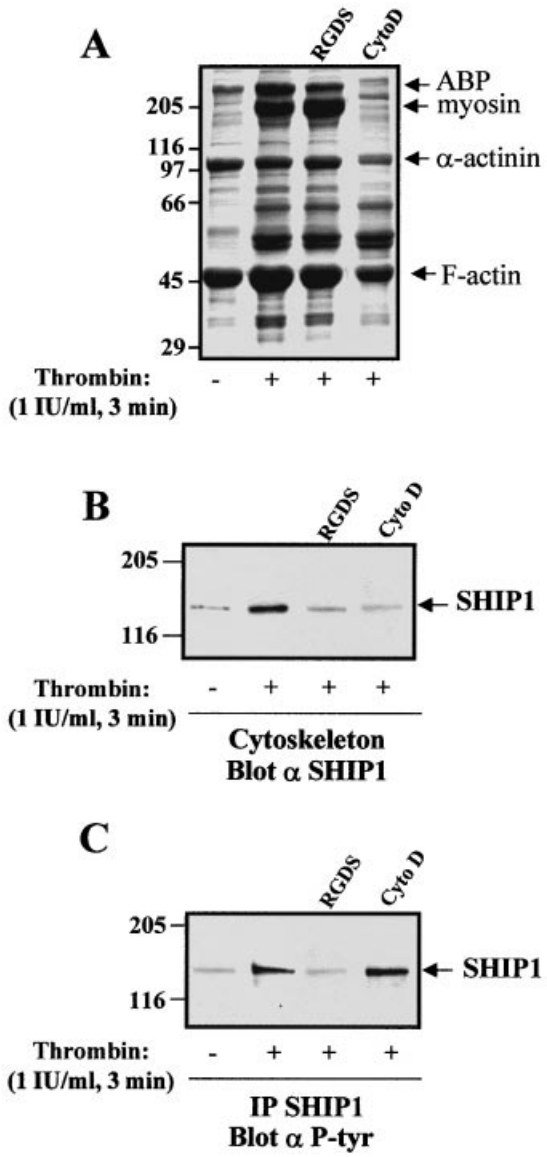

Figure 4 Actin polymerization is not required for tyrosine phosphorylation of SHIP1

Cytoskeletons were extracted from resting platelets or from platelets stimulated by 1 i.u./ml thrombin for 3 min, preincubated or not with 10 μM cytochalasin D or 200 μg/ml RGDS, as indicated in the Materials and methods section. Cytoskeletal proteins, extracted from the same number of platelets (5×10^8), were separated and detected by SDS/PAGE [7.5% (w/v) gel] stained with Coomassie Blue. Actin and the major actin-binding proteins are identified at the right (**A**). Cytoskeletal proteins were subjected to immunoblotting with anti-SHIP1 antibody (α SHIP1) (**B**). Anti-SHIP1 immunoprecipitates from resting platelets or from platelets or activated by 1 i.u./ml thrombin for 3 min, pretreated or not with 10 μM cytochalasin D or 200 μg/ml RGDS, were subjected to immunoblotting with 4G10 anti-phosphotyrosine antibody (α P-tyr) (**C**). The positions of molecular mass markers are indicated (in kDa) at the left of each panel.

skeleton and only weakly inhibited the actin assembly (Figure 4A).

DISCUSSION

Several tyrosine kinases, including pp60$^{c\text{-}src}$, Syk and FAK, have been shown to be recruited and activated downstream integrin engagement in platelets [5]. The activation of pp60$^{c\text{-}src}$ and Syk is an early event after integrin engagement and is independent of actin polymerization, whereas the activation of FAK occurs late in integrin signalling and is dependent on actin polymerization [30]. Our results show that pp60$^{c\text{-}src}$ is constitutively associated with SHIP1 and that this pool of pp60$^{c\text{-}src}$ becomes tyrosine-phosphorylated after stimulation by thrombin. This tyrosine phosphorylation is in agreement with the enhanced protein kinase activity measured in SHIP1 immunoprecipitates. On the basis of the effect of a pharmacological inhibitor of the Src family, PP-1, the results suggest that pp60$^{c\text{-}src}$ can indeed phosphorylate SHIP1 both *in vitro* and *in vivo*. Moreover, a third partner, p64, not yet identified, immunoprecipitates with SHIP1. This protein is weakly tyrosine-phosphorylated under resting conditions and becomes strongly tyrosine-phosphorylated on stimulation with thrombin. An unknown protein of this molecular mass with the same pattern of tyrosine phosphorylation in resting and activated platelets has been observed previously [31]. The ability to immunoprecipitate p64 in relatively large amounts with SHIP1 could facilitate the identification of this protein in future experiments.

Interestingly, in a glutathione S-transferase fusion protein pull-down experiment, Wisniewski et al. [32] recently showed a specific interaction of the SH3 domain of Src with SHIP1. This interaction is consistent with SHIP1's bearing proline-rich stretches in its C-terminal tail. The nature of the interaction mediated by the SH3 domain and proline-rich regions might explain the constitutive association between pp60$^{c\text{-}src}$ and SHIP1.

Our results also show that SHIP1 does not require tyrosine phosphorylation for its interaction with the cytoskeletal and signalling proteins associated with the cytoplasmic domain of integrins. This suggests that SHIP1 is first recruited to the vicinity of integrin cytoplasmic domains, probably as a complex with pp60$^{c\text{-}src}$ and the unknown p64, and then becomes phosphorylated by a Src-kinase-dependent mechanism. Among the Src-kinases, pp60$^{c\text{-}src}$ is the best candidate because it is constitutively associated with SHIP1, relocates to the cytoskeleton on integrin engagement and is rapidly activated by αIIbβ3, the major platelet integrin [33]. Assembly and reorganization of the actin cytoskeleton are required for several integrin signalling pathways [5,30]. Interestingly, the tyrosine phosphorylation of SHIP1 does not require actin polymerization. The activation of pp60$^{c\text{-}src}$ by αIIbβ3 has recently been shown to be independent of actin polymerization as well [30], and to be regulated by events proximal to the integrin tail. Our results also indicate that SHIP1 does not associate with newly synthesized filamentous actin structures but instead interacts with proximal integrin-linked multiprotein complexes formed independently of actin polymerization. These complexes become strongly associated with the reorganized actin cytoskeleton during platelet aggregation and then sediment with the Triton X-100-insoluble material at 12000 ***g*** [6].

It is not yet known how SHIP1 is recruited to the integrin-linked multiprotein complexes, but one can postulate that the potentially tyrosine-phosphorylated motif of the β3 cytoplasmic tail of αIIbβ3 might interact with the SH2 domain of SHIP1. This attractive hypothesis is currently under investigation. Moreover, an interaction of SHIP1 with the ITAM-like motif of another adhesion receptor, PECAM1, has recently been described [12]. PECAM is also present in platelets, can regulate the integrin activation and is re-distributed to the cytoskeleton on platelet aggregation [13]. A functional interaction between SHIP1 and PECAM1 is therefore conceivable in the proximity of αIIbβ3 clusters.

The function of SHIP1 downstream of integrins remains unknown. Its contribution to the integrin-dependent accumu-

lation of PtdIns(3,4)P_2, which then has a role in the strengthening of irreversible aggregation [18,19], is difficult to assess because other metabolic pathways are involved in the synthesis of this particular lipid. Moreover, other PtdIns(3,4,5)P_3 5-phosphatases have been described in platelets [34] and might also contribute to the transformation of PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2. This would be in agreement with the fact that SHIP1-deficient mice do not die from haemostatic complications [35]. In addition, once recruited and tyrosine-phosphorylated, SHIP1 might also have a role as a docking protein, allowing the formation of suitable signalling complexes at the proximity of integrin clusters. Clearly, further studies will be required to elucidate the function of SHIP1, and possibly SHIP2, in activated human blood platelets.

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