Biochemical and Biophysical Research Communications **256,** 114–120 (1999)
Article ID bbrc.1999.0295, available online at http://www.idealibrary.com on IDEAL®

Signal Transduction Pathways Mediated by Glycoprotein Ia/IIa in Human Platelets: Comparison with Those of Glycoprotein VI

Katsue Inoue,* Yukio Ozaki,*,[1] Kaneo Satoh,* Yi Wu,* Yutaka Yatomi,* Yongchol Shin,† and Takashi Morita†

**Department of Clinical and Laboratory Medicine, Yamanashi Medical University, Shimokato 1110 Tamaho, Nakakoma, Yamanashi 409-3898, Japan; and †Department of Biochemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo, Japan*

Received January 29, 1999

Human platelets were activated either by glycoprotein (GP) Ia/IIa agonist (rhodocytin) or by a GPVI agonist (collagen-related peptide, CRP), and the intracellular signal transduction pathways were compared in the presence of various inhibitors. Rhodocytin isolated from *Calloselasma rhodostoma* venom was verified as a GPIa/IIa agonist, based on the inhibitory effects of three mAbs directed against GPIa. Platelet activation mediated by GPIa/IIa led to overt platelet aggregation, elevation of intracellular Ca^{2+}, and tyrosine phosphorylation of several proteins, similar to that of GPVI. $p72^{syk}$ and phospholipase Cγ2 (PLCγ2) tyrosine phosphorylation were also observed with GPIa/IIa-mediated platelet aggregation, although they peaked slightly later than that of GPVI. In contrast to GPVI-mediated platelet activation, most of these phenomena induced by GPIa/IIa activation were markedly suppressed by acetylsalicylic acid (ASA) or cytochalasin D. These findings suggest that the requirements for thromboxane A_2 (TXA_2) production and actin polymerization, which are the characteristics of collagen-induced platelet activation, are derived from the GPIa/IIa-mediated signal transduction, but not from that of GPVI.

Key Words: **platelet; collagen; glycoprotein Ia/IIa; glycoproteinVI; snake venom; rhodocytin.**

Collagen is one of the major extracellular matrix proteins present in blood vessel subendothelium. Platelets adhere to collagen fibers exposed at the site of damage to the endothelial lining and become activated through specific membrane receptors for collagen. Collagen-induced platelet activation has several characteristic properties, distinct from other agonists. First, collagen-induced platelet activation is known to be particularly sensitive to ASA, which inhibits the production of thromboxane A_2 and cytochalasins, which interfere with actin polymerization (1, 2). Second, collagen and FcγRII cross-linking, but neither thrombin nor thromboxane A_2, elicit PLCγ2 activation (3, 4). However, which putative collagen receptor (see below) is responsible for these properties has not been determined.

Many candidates have been proposed for putative collagen receptors on platelet membranes. Of these, GPIa/IIa and GPVI have now established their roles as collagen receptors (5); It has been reported that human blood platelets which showed no response to collagen lacked the surface expression of GPIa, indicating that GPIa/IIa is a putative collagen receptor (6–8). Several groups reported that platelets deficient in GPVI lacked collagen-induced aggregation, indicating that GPVI is also a collagen receptor (9–11).

Collagen-related peptide (CRP) containing repeats of the Gly-Pro-Hyp sequence (12–15), and convulxin, which is a C-type lectin obtained from the tropical rattlesnake venom, activate platelets, interacting with GPVI (16, 17). These proteins have offered great help for investigating the signal transduction pathways mediated by GPVI; GPVI-related platelet activation is mediated via a tyrosine kinase-dependent pathway, resulting in phosphorylation of the Fc receptor γ-chain, binding of $p72^{syk}$ to the γ chain, which induces the phosphorylation of PLCγ2 (18–20). PLCγ2 then induces intracellular Ca^{2+} mobilization via the inositol phospholipid-dependent pathway.

On the other hand, there have been few GPIa/IIa agonists appropriate for investigating the GPIa/IIa-related signal transduction pathway. JBS2, an anti-μ_2 integrin mAb stimulated tyrosine phosphorylation of PLCγ2 in human platelets. However, this phosphory-

[1] To whom correspondence should be addressed. Fax: +81 552 73 6713. E-mail: yozaki@res.yamanashi-med.ac.jp.

0006-291X/99 $30.00

lation is dependent on FcγRII as $F(ab')_2$ fragments are inactive (21). Aggretin purified from the *Calloselasma rhodostoma* venom, is reported to induce platelet aggregation acting as a GPIa/IIa agonist, although its functional property has not been fully elucidated (22). Therefore, although it has been well established that GPIa/IIa plays a major role in platelet adhesion to collagen (5, 23), it remains unclear what signals GPIa/IIa mediates, which lead to platelet activation.

Recently, we have isolated and characterized a functionally novel platelet agonist, designated as rhodocytin, from the *Calloselasma rhodostoma* venom. Rhodocytin belongs to the heterodimeric C-type lectin family and induces platelet aggregation independently of glycoprotein Ib (24).

In this study, we present evidence to indicate that rhodocytin induces platelet activation by interacting with GPIa/IIa. Using this venom as a GPIa/IIa agonist, we have attempted to characterize the GPIa/IIa-mediated signal transduction pathway in platelets, compared with the GPVI-mediated pathway, using CRP.

MATERIALS AND METHODS

Materials. Rhodocytin was purified from the venom of *Calloselasma rhodostoma* as described previously (24). Collagen-related peptide (CRP) was a generous gift from Dr. M. Moroi (Department of Protein Biochemistry, Institute of Life Science, Kurume University, Fukuoka, Japan). Anti-integrin alpha2 mAb (6F1) was kindly provided by Dr. B. S. Coller (Mt. Sinai Medical Center, New York, NY). B6H12, anti-IAP (integrin-associated protein) mAb was kindly donated by Dr. K. Fujimura (Department of Hematology and Oncology, Hiroshima University, Japan). The following materials were obtained from the indicated suppliers: Anti-$p72^{syk}$ mAb, (Wako Pure Chemical Industries, Ltd., Tokyo, Japan); anti-PLCγ2 polyclonal antibody (pAb) (Santa Cruz, CA); anti-phosphotyrosine mAb (PY20) (Transduction Laboratories, Lexington, KY); anti-phosphotyrosine mAb (4G10), anti-integrin alpha2 mAb (A2-IIE10) (Upstate Biotechnology, Inc. Lake Placid, NY); anti-GPIa/IIa mAb (Gi9) (Immunotech S. A. Marseilles, France).

Platelet preparation, platelet aggregation, and $[Ca^{2+}]_i$ measurement. Platelets obtained from healthy donors were washed, and suspended in a Hepes–Tyrode's buffer. In some experiments, platelet-rich plasma was incubated with 1 mM ASA for 30 min before washing. When indicated, washed platelets were preincubated with 10 μg/ml of cytochalasin D, the indicated concentration of A2-IIE10, 6F1, Gi9 or B6H12 for 5 min at 37°C. Platelet aggregation was measured by the conventional turbidimetric method. In some experiments, intracellular Ca^{2+} mobilization was measured by the Fura-2 method as described previously (25). Fura-2 fluorescence was measured with an excitation wave length alternating every 0.5 s from 340 to 380 nm, and the emission wavelength was set at 510 nm. The $[Ca^{2+}]_i$ values were determined from the ratio of Fura-2 fluorescence intensity at 340 and 380 nm excitation.

Immunoprecipitation and Western blotting. Washed platelets pretreated with or without various inhibitors were stimulated with the indicated agonist. Reactions were terminated with lysis buffer, and after sonication, the supernatant was isolated by centrifugation. PLCγ2 and $p72^{syk}$ were immunoprecipitated from the supernatant using anti-PLCγ2 and $p72^{syk}$ antibodies. To identify phosphotyrosine-containing platelet proteins, reactions terminated with an equal volume of Laemmli sodium dodecyl sulfate reducing buffer (25). The immunoprecipitates or platelet proteins were subjected to electrophoresis and western blotting, as described previously (25).

RESULTS

Inhibition by Anti-GPIa mAbs of Platelet Aggregation Induced by Rhodocytin

As we described previously, rhodocytin at concentrations higher than 5 nM induced aggregation of washed platelets with a long lag time, as well as platelets in platelet-rich plasma. Aggregation started with a lag time which became shorter with higher concentrations of rhodocytin (Fig. 1A, a–d). However, even at highest concentrations of rhodocytin tested (30 nM), there remained a distinct lag time.

We have already shown that rhodocytin elicits platelet aggregation independently of GPIb (24). Since the presence of a long lag time before aggregation, the susceptibility to ASA and cytochalasin D treatment are all reminiscent of collagen-induced platelet aggregation, we then evaluated the effects of mAbs that block collagen-induced platelet adhesion on rhodocytin-induced platelet aggregation. 6F1, A2-IIE10, and Gi9 are mAbs directed against GPIa, the former two mAbs almost completely blocked collagen-induced platelet aggregation, while the latter only partially inhibited collagen-induced platelet aggregation even at a concentration of 100 μg/ml (data not shown). 6F1 at 100 μg/ml and Gi9 at 50 μg/ml completely inhibited platelet aggregation induced by rhodocytin in most cases (Fig. 1B), whereas the inhibition was partial in some cases. On the other hand, rhodocytin-induced platelet aggregation was completely inhibited by A2-IIE10 at 25 μg/ml (Fig. 1A, e, 1B), the concentration which also inhibits collagen-induced platelet aggregation (data not shown). None of these anti-GPIa antibodies inhibited platelet aggregation induced by CRP or thrombin (data not shown). B6H12, a mAb directed to the integrin-associated protein, was without effects on rhodocytin-induced platelet aggregation even at a concentration of 100 μg/ml (Fig. 1B). These findings suggest that rhodocytin activates platelets by interacting with GPIa/IIa.

Intracellular Ca^{2+} Mobilization and Protein–Tyrosine Phosphorylation Induced by Rhodocytin

We then evaluated the signal transduction pathways mediated by GPIa/IIa using rhodocytin. Ten nanomolar rhodocytin elevated the intracellular Ca^{2+} concentration with a distinct lag time and a gentle slope in the presence and absence of extracellular Ca^{2+} (Fig. 2A). It is well known that tyrosine kinases play an important role in platelet activation, especially of collagen stimulation. Similar to collagen-induced activation (25), rhodocytin stimulation (Fig. 2B) resulted in tyrosine

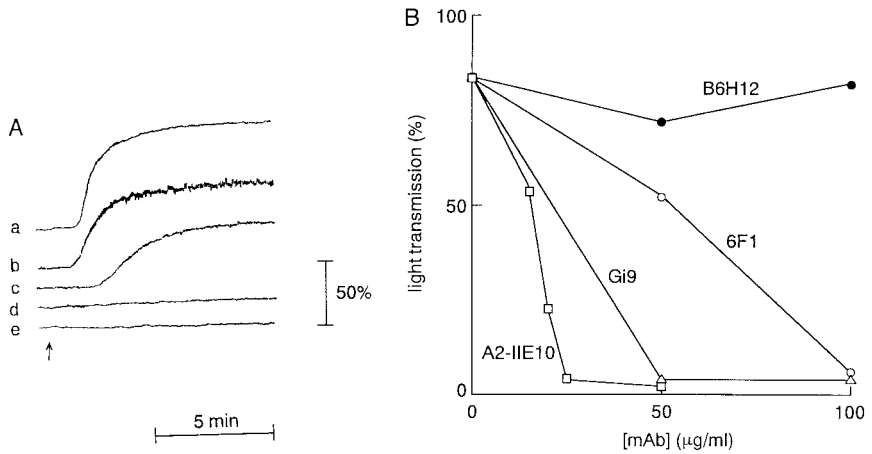

FIG. 1. Platelet aggregation induced by rhodocytin and its inhibition by anti-GPIa mAbs. In A, washed platelets were stimulated with 30 nM (a), 10 nM (b), 5 nM (C), or 2.5 nM (d) rhodocytin, added at the time indicated by an arrow. Platelets were pretreated with 25 μg/ml of A2-IIE10 for 5 min, then activated with 10 nM rhodocytin (e). The ordinate represents percentage changes in light transmission. The data are representative of at least three experiments. In B, platelets were preincubated with a vehicle solution or the indicated concentrations of A2-IIE10, 6F1, Gi9 or B6H12 for 5 min, then stimulated with 10 nM rhodocytin. Platelet aggregation was evaluated by the maximum changes in light transmission within 10 min.

phosphorylation of 64-, 70- to 75-, 97-, and 125-kDa proteins in platelets, which peaked at about 1–5 min after activation. Since we reported that the most heavily tyrosine-phosphorylated 70- to 75-kDa band in collagen-stimulated platelets includes $p72^{syk}$ (25), we checked whether rhodocytin also induced $p72^{syk}$ tyrosine phosphorylation. Immunoprecipitation of $p72^{syk}$ with an anti-$p72^{syk}$ antibody, followed by Western blotting with an anti-phosphotyrosine antibody, revealed that the autophosphorylated level of $p72^{syk}$ increased, although slightly, 1–2 min after rhodocytin stimulation (Fig. 2C). PLCγ2 lies downstream of $p72^{syk}$ activation in platelets (26). In platelets, collagen and FcγRII cross-linking, but not thrombin or thromboxane A_2, elicit PLCγ2 activation, which can be assessed by the level of its tyrosine phosphorylation (3, 4). Hence, we evaluated PLCγ2 tyrosine phosphorylation in platelet activation induced by rhodocytin. Rhodocytin induced PLCγ2 tyrosine phosphorylation, which peaked 2 minutes after stimulation (Fig. 2D).

All these findings suggest that GPIa/IIa-mediated signals involve $p72^{Syk}$ and PLCγ2 tyrosine phosphorylations and subsequent Ca^{2+} mobilization, which then leads to platelet aggregation.

Effects of Various Inhibitors on Platelet Activation Induced by Rhodocytin or CRP

The findings hitherto obtained demonstrate that GPIa/IIa-mediated signals induce platelet aggregation, intracellular Ca^{2+} mobilization, activation of $p72^{syk}$ and of PLCγ2. We then compared characteristics of GPIa/IIa-mediated platelet activation and that of GPVI-mediated platelet activation, using rhodocytin and CRP.

Platelet aggregation and intracellular Ca^{2+} mobilization. Platelets pretreated with 1 mM ASA for 30 min did not aggregate in response to rhodocytin (Fig. 3A, a, b). Rhodocytin did not induce aggregation of platelets incubated with 10 μg/ml of cytochalasin D for 5 min (Fig. 3A, a, c). Inhibitors such as 1 mM ASA and 10 μg/ml of cytochalasin D also blocked rhodocytin-induced intracellular Ca^{2+} mobilization (Fig. 3B, a–c), whereas CRP-induced platelet aggregation (Fig. 3A, d–f) were not blocked by ASA or by cytochalasin D. Cytochalasin D slightly inhibited the initial slope on the light transmission curve, but did not affect the maximum light transmission. Intracellular Ca^{2+} mobilization induced by CRP was not inhibited by ASA and cytochalasin D (Fig. 3B, d–f).

Tyrosine phosphorylation of $p72^{syk}$ and PLCγ2. We found that $p72^{syk}$ was slightly tyrosine-phosphorylated 1-2 min after rhodocytin stimulation. This phosphorylation was markedly suppressed by ASA, and the inhibition was complete with cytochalasin D (Fig. 4A). It is of interest that cytochalasin D totally inhibited the resting level of $p72^{syk}$ tyrosine phosphorylation, as well as the change after rhodocytin stimulation. In contrast, CRP induced rapid and intense tyrosine phosphorylation of $p72^{syk}$ (15 s after stimulation), which was not blocked by ASA (Fig. 4B). The CRP-induced tyrosine phosphorylation of $p72^{syk}$ was slightly inhibited by cytochalasin D. However, the inhibition was far from complete, as seen with rhodocytin. With rhodocytin stimulation, PLCγ2 phosphorylation occurred 2 min after stimulation. It was markedly suppressed by ASA and was completely inhibited by cytochalasin D (Fig. 4C). In contrast, CRP induced PLCγ2 tyrosine-phosphorylation earlier than rhodocytin and its phos-

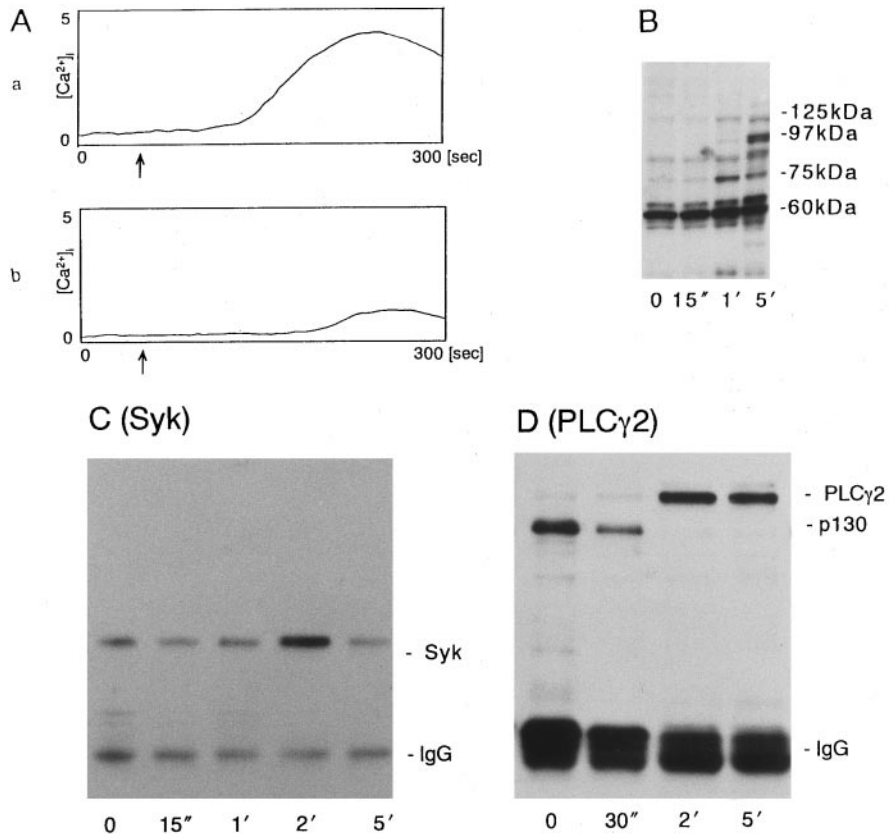

FIG. 2. Intracellular Ca^{2+} mobilization, protein-tyrosine phosphorylation (PTP) and tyrosine phosphorylation of $p72^{syk}$ and PLCγ2 induced by rhodocytin. In A, platelets were incubated with buffer containing 1 mM $CaCl_2$ (a) or 200 μM EGTA (b) for 5 min, and then stimulated with 10 nM rhodocytin, added at the time indicated by arrows. The $[Ca^{2+}]_i$ elevation was monitored as changes in fura-2 fluorescence for 300 s. The ordinate represents the ratio of fura-2 fluorescence. In B, platelets were activated by 20 nM rhodocytin for the indicated periods and reactions were stopped with Laemmli sample buffer. Platelet proteins were applied to SDS–PAGE, and tyrosine-phosphorylated proteins were detected with Western blotting using anti-phosphotyrosine mAbs (4G10 *plus* PY20). In C and D, platelets were activated by 20 nM rhodocytin and reactions were terminated by adding lysis buffer after the indicated periods. Platelet proteins associated with $p72^{syk}$ (C) and PLCγ2 (D) were immunoprecipitated with anti-$p72^{syk}$ mAb and anti-PLCγ2 pAb, respectively. The samples were then Western-blotted using the anti-phosphotyrosine mAbs. The data are representative of at least three experiments.

phorylation was hardly inhibited by ASA, while slight inhibition was observed in cytochalasin D pretreatment (Fig. 4D).

DISCUSSION

We showed that rhodocytin, purified from the *Calloselasma rhodostoma* venom, induces platelet aggregation independently of GPIb (24). We have extended investigation to suggest that rhodocytin activates platelets by interacting with GPIa/IIa, which is one of the major collagen receptors.

The evidence is derived from the effects of anti-collagen receptor mAbs. Platelet aggregation induced by rhodocytin was completely blocked by three kinds of anti-GPIa mAbs. Of these, A2-IIE10 inhibited rhodocytin-induced platelet aggregation at concentrations from 20 to 25 μg/ml, which fall in the same concentration range needed for inhibition of collagen-induced platelet aggregation. Platelet aggregation induced by rhodocytin was inhibited by 6F1 at concentrations which are substantially higher than for collagen. The difference in the concentrations among anti-GPIa mAbs is probably due to the differences in the sites of interaction between GPIa/IIa and collagen, and between GPIa/IIa and rhodocytin. A2-IIE10 may recognize an epitope closely shared by collagen and rhdocytin, while 6F1 may bind to a site more related to the collagen binding site.

Watson and his group, and Okuma and his group have already shown that GPVI-related platelet activation is mediated via a tyrosine kinase-dependent pathway, resulting in the phosphorylation of PLCγ2 (18–20). On the other hand, relatively little has been elucidated regarding the GPIa/IIa-mediated signal transduction system. Kehrel *et al.* demonstrated that GPVI-deficient platelets bound to fibrinogen in response to collagen, but not to CRP. Since collagen but

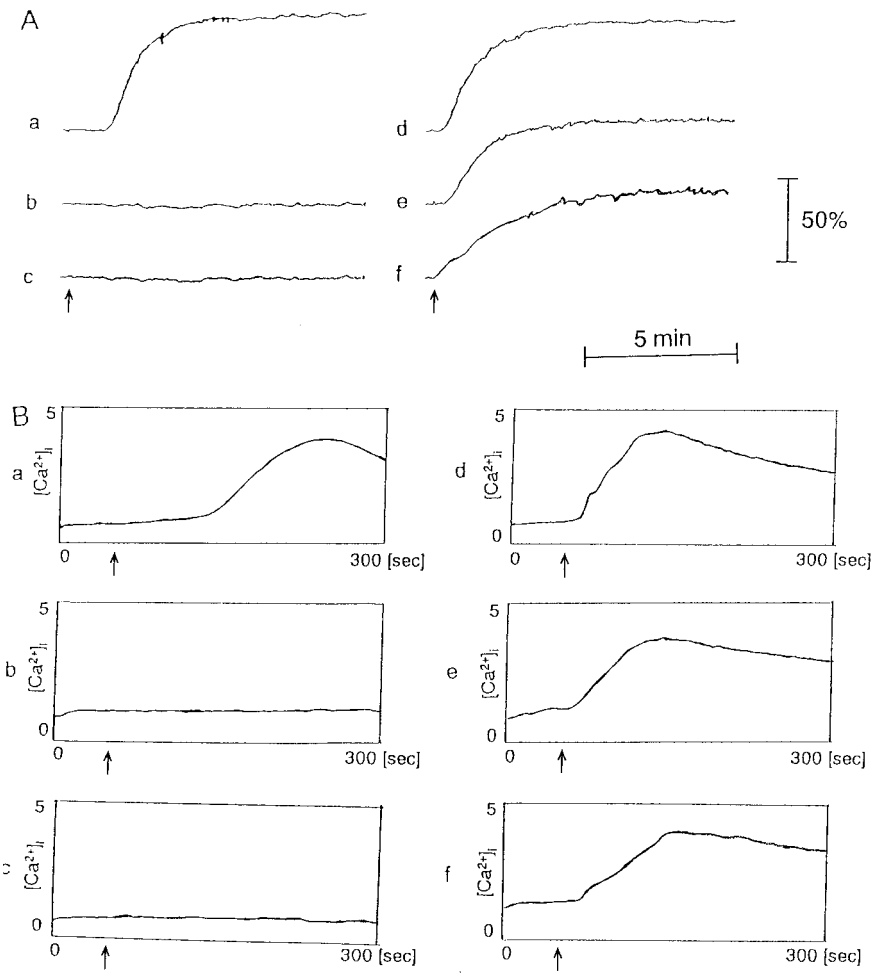

FIG. 3. Effects of ASA and cytochalasin D on platelet aggregation and intracellular Ca^{2+} mobilization induced by rhodocytin or CRP. Platelets were preincubated with a vehicle solution (a, d), 1 mM ASA (b, e) and 10 μg/ml of cytochalasin D (c, f) as described in Materials and Methods, then stimulated with 10 nM rhodocytin (a–c) or 0.5 μg/ml CRP (d–f). In A, platelet aggregation was measured by the conventional turbidmetric method. The ordinate represents percentage changes in light transmission. In B, the $[Ca^{2+}]_i$ elevation was monitored as changes in fura-2 fluorescence for 300 s. The ordinate represents the ratio of fura-2 fluorescence. The data are representative of at least three experiments.

not CRP contains the binding sites for GPIa/IIa, it is suggested that GPIa/IIa also induces certain intracellular signals leading to activation of GPIIb/IIIa (13). However, what intracellular signal is related with GPIa/IIa itself, leading to platelet activation, has been unknown, for few GPIa/IIa agonists have been available to date. To the best of our knowledge, this is the first report which demonstrates that GPIa/IIa mediates tyrosine phosphorylation of several proteins, syk and PLCγ2 activation.

Collagen-induced platelet activation has several characteristic properties, distinct from other agonists. First, collagen-induced platelet activation, especially that induced by low concentrations of collagen ($<$10 μg/ml), is known to be particularly sensitive to ASA. Second, cytochalasins, which interfere with actin polymerization, blocks collagen-induced platelet activation such as aggregation, intracellular Ca^{2+} mobilization and arachidonic acid release (1, 2). In contrast, these parameters of platelet activation induced by soluble agonists such as thrombin or ADP are only slightly modified by cytochalasins. Third, collagen and FcγRII cross-linking, but neither thrombin nor thromboxane A_2, elicit PLCγ2 activation (3, 4). However, which putative receptor (GPIa/IIa, GPVI, or others) is responsible for these properties has not been determined.

We also investigated the differences in activation signals between GPIa/IIa- and GPVI- mediated platelet activation. We showed that ASA and cytochalasin D completely inhibited platelet aggregation, intracellular Ca^{2+} mobilization, and tyrosine-phosphorylation of $p72^{syk}$ and PLCγ2 mediated by GPIa/IIa, whereas GPVI-related platelet activation was almost totally resistant to them. As we mentioned above, collagen-

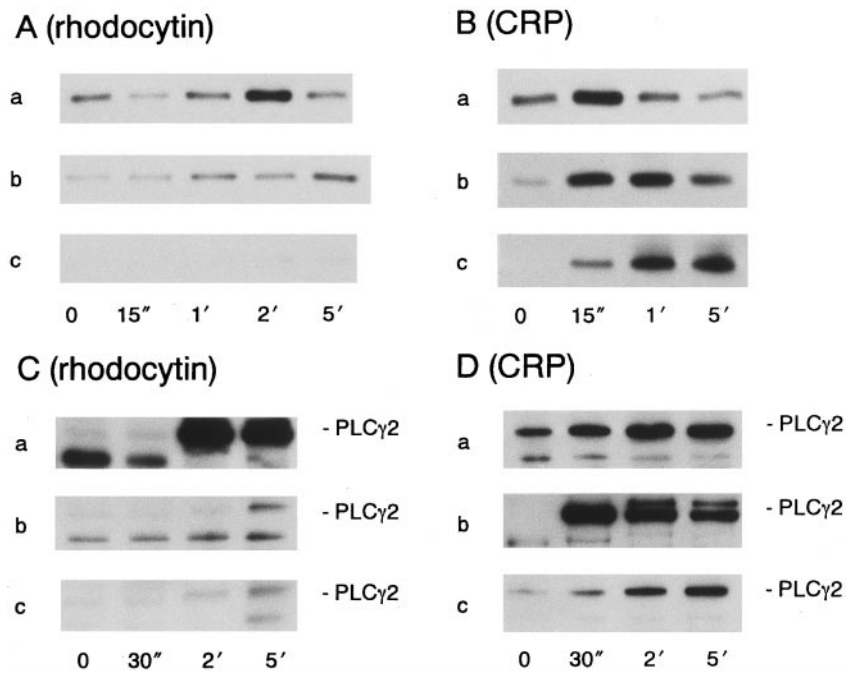

FIG. 4. Effects of ASA and cytochalasin D on p72^{syk} and PLCγ2 tyrosine phosphorylation induced by rhodocytin or CRP. Platelets were incubated with a vehicle solution (a), 1 mM ASA (b) or 10 μg/ml of cytochalasin D (c) as described under Materials and Methods, then activated by 20 nM rhodocytin (A, C) or 0.5 μg/ml of CRP (B, D) for indicated periods of time. Reactions were terminated with lysis buffer, and platelet proteins associated with p72^{syk} or PLCγ2 were immunoprecipitated with anti-p72^{syk} mAb (A, B) or anti-PLCγ2 pAb (C, D). The sample was then Western-blotted with the anti-phosphotyrosine mAbs. The data are representative of at least three experiments.

induced platelet activation is known to be particularly sensitive to ASA and cytochalasins (1, 2). These properties, which characterize collagen-induced platelet activation, can be ascribed to the GPIa/IIa-related signal transduction pathway. Our findings also suggest that TXA_2 production and actin polymerization plays an important role in the GPIa/IIa-mediated pathway, and the requirement of actin polymerization may further imply that dimerization or clustering of GPIa/IIa is necessary for platelet activation.

In conclusion, we have found that GPIa/IIa stimulation induces p72^{syk} and PLCγ2 activation, which is similar to GPVI stimulation. However, distinct from GPVI-mediated platelet activation, GPIa/IIa-mediated platelet activation was sensitive to ASA and cytochalasin D. These findings suggest that the requirement for TXA_2 production and actin polymerization, which is the characteristic property of collagen-induced platelet activation, is derived from the GPIa/IIa-mediated signal transduction pathway, but not from that of GPVI.

ACKNOWLEDGMENTS

We are grateful to Drs. M. Moroi, B. S. Coller, and K. Fujimura for providing CRP, 6F1, and B6H12, respectively.

REFERENCES

1. Nakano, T., Hanasaki, K., and Arita, H. (1989) *J. Biol. Chem.* **264,** 5400–5406.
2. Peerschke, E. I., and Zucker, M. B. (1980) *Thromb. Haemost.* **43,** 58–60.
3. Blake, R. A., Schieven, G. L., and Watson, S. P. (1994) *FEBS Lett.* **353,** 212–216.
4. Blake, R. A., Asselin, J., Walker, T., and Watson, S. P. (1994) *FEBS Lett.* **342,** 15–18.
5. Watson, S. P., and Gibbins, J. (1998) *Immunol. Today* **19,** 260–264.
6. Nieuwenhuis, H. K., Akkerman, J. W., Houdijk, W. P., and Sixma, J. J. (1985) *Nature* **318,** 470–472.
7. Handa, M., Watanabe, K., Kawai, Y., Kamata, T., Koyama, T., Nagai, H., and Ikeda, Y. (1995) *Thromb. Haemost.* **73,** 521–528.
8. Kehrel, B., Balleisen, L., Kokott, R., Mesters, R., Stenzinger, W., Clemetson, K. J., and Loo, J. (1988) *Blood* **71,** 1074–1078.
9. Arai, M., Yamamoto, N., Moroi, M., Akamatsu, N., Fukutake, K., and Tanoue, K. (1995) *Br. J. Haematol.* **89,** 124–130.
10. Moroi, M., Jung, S. M., Okuma, M., and Shinmyozu, K. (1989) *J. Clin. Invest.* **84,** 1440–1445.
11. Ryo, R., Yoshida, A., Sugano, W., Yasunaga, M., Nakayama, K., Saigo, K., Adachi, M., Yamaguchi, N., and Okuma, M. (1992) *Am. J. Hematol.* **39,** 25–31.
12. Verkleij, M. W., Morton, L. F., Knight, C. G., Groot, P. G., Barnes, M. J., and Sixma, J. J. (1998) *Blood* **91,** 3808–3816.

13. Kehrel, B., Wierwille, S., Clemetson, K. J., Anders, O., Steiner, M., Knight, C. G., Farndale, R. W., Okuma, M., and Barnes, M. J. (1998) *Blood* **91,** 491–499.
14. Morton, L. F., Hargreaves, P. G., Farndale, R. W., Young, R. D., and Barnes, M. J. (1995) *Biochem. J.* **306,** 337–344.
15. Sugiyama, T., Okuma, M., Ushikubi, F., Sensaki, S., Kanaji, K., and Uchino, H. (1987) *Blood* **69,** 1712–1720.
16. Jandrot-Perrus, M., Lagrue, A. H., Okuma, M., and Bon, C. (1997) *J. Biol. Chem.* **272,** 27035–27041.
17. Polgar, J., Clemetson, J. M., Kehrel, B. E., Wiedemann, M., Magnenat, E. M., Wells, T. N. C., and Clemetson, K. J. (1997) *J. Biol. Chem.* **272,** 13576–13583.
18. Gibbins, J. M., Okuma, M., Farndale, R., Barnes, M., and Watson, S. P. (1997) *FEBS Lett.* **413,** 255–259.
19. Melford, S. K., Turner, M., Briddon, S. J., Tybulewicz, V. L., and Watson, S. P. (1997) *J. Biol. Chem.* **272,** 27539–27542.
20. Tsuji, M., Ezumi, Y., Arai, M., and Takayama, H. (1997) *J. Biol. Chem.* **272,** 23528–23531.
21. Keely, P. J., and Parise, L. V. (1996) *J. Biol. Chem.* **271,** 26668–26676.
22. Huang, T. F., Liu, C. Z., and Yang, S. H. (1995) *Biochem. J.* **309,** 1021–1027.
23. Sixma, J. J., Zanten, G. H., Huizinga, E. G., Plas, R. M., Verkley, M., Wu, Y. P., Gros, P., and Groot, P. G. (1997) *Thromb. Haemost.* **78,** 434–438.
24. Shin, Y., Morita, T. (1998) *Biochem. Biophys. Res. Commun.* **245,** 741–745.
25. Asazuma, N., Yatomi, Y., Ozaki, Y., Qi, R., Kuroda, K., Satoh, K., and Kume, S. (1996) *Thromb. Haemost.* **75,** 648–654.
26. Poole, A., Gibbins, J. M., Turner, M., Vugt, M. J., Winkel, J. G., Saito, T., and Tybulewicz, V. L. S. P. W. (1997) *EMBO J.* **16,** 2333–2341.