Platelets play critical roles in hemostasis and thrombosis through their aggregation following activation of integrin $\alpha_{IIb}\beta_3$. However, the molecular mechanism of the integrin activation inside platelets remains largely unknown. Pharmacological experiments have demonstrated that protein kinase C (PKC) plays an important role in platelet aggregation. Because PKC inhibitors can have multiple substrates and given that non-PKC-phorbol ester-binding signaling molecules have been demonstrated to play important roles, the precise involvement of PKC in cellular functions requires re-evaluation. Here, we have established an assay for analyzing the $\text{Ca}^{2+}$-induced aggregation of permeabilized platelets. The aggregation of platelets was inhibited by the addition of the arginine-glycine-aspartate-serine peptide, an integrin-binding peptide inhibitor of $\alpha_{IIb}\beta_3$, suggesting that the aggregation was mediated by the integrin. The aggregation was also dependent on exogenous ATP and platelet cytosol, indicating the existence of essential cytosolic factors required for the aggregation. To examine the role of PKC in the aggregation assay, we immunodepleted PKCa and $\beta$ from the cytosol. The PKC-depleted cytosol lost the aggregation-supporting activity, which was recovered by the addition of purified PKCs. Furthermore, the addition of purified PKCa in the absence of cytosol did not support the aggregation, whereas the cytosol containing less PKC supported it efficiently, suggesting that additional factors besides PKC would also be required. Thus, we directly demonstrated that PKC is involved in the regulation of $\text{Ca}^{2+}$-induced platelet aggregation.

Platelets play critical roles in hemostasis and thrombosis through aggregation following activation of integrin $\alpha_{IIb}\beta_3$. Although $\alpha_{IIb}\beta_3$ of platelets in the resting stage does not bind fibrinogen or von Willebrand factors (vWF), the activation of platelets results in conformation changes, which allow $\alpha_{IIb}\beta_3$ to bind these ligands (1–3). When fibrinogen or vWF binds to $\alpha_{IIb}\beta_3$, the ligand-occupied integrin signals downstream and stabilizes platelet aggregation through reorganization of the actin cytoskeletal network and the release of bioactive substances stored in the granules (1–3). Thus, the process of platelet activation and aggregation consists of a series of orchestrated responses (1–8). However, the molecular mechanisms that underlie this process remain unclear because it is difficult to use molecular biology and biochemistry in platelets that do not synthesize new proteins. To overcome this difficulty, semi-intact assays using permeabilized platelets have been established for the study of granule secretion (4–8). Although some semi-intact aggregation assays have now been developed, it has been difficult to demonstrate a cytosol dependence in these experiments (8).

Protein kinase C (PKC) family members are important signaling molecules regulating many cellular functions (9, 10). Among the family members, conventional PKCs (cPKC), which include PKCa, $\beta$, $\beta$I, and $\gamma$, have regulatory $\text{Ca}^{2+}$- and phorbol ester-binding domains (9, 10). The involvement of cPKCs in the cellular functions has been analyzed mainly pharmacologically using cell-permeable small compounds of inhibitors and stimulators such as phorbol esters. However, these experiments are somewhat indirect because 1) no inhibitors have absolute specificity and 2) important signaling molecules other than PKCs have also been demonstrated to contain phorbol-ester-binding C1-domains, which were first identified in cPKC (9, 10). For example, Ras-guanyl nucleotide-releasing protein (11, 12) contains the C1 domain at its C terminus and acts as a stimulator for small GTPase Ras involved in the regulation of cell growth. Chimerin (13, 14) also contains a C1 domain and acts as a GTPase-activating protein of small GTPase Rac involved in the regulation of cytoskeletal reorganization. Thus, the effects of phorbol esters could be through multiple pathways. Therefore, it is important to re-evaluate and demonstrate the involvement of PKCs in certain cellular functions in a direct fashion (15).

In platelets, PKC has been considered to play important roles in aggregation (16, 17) and granule secretion (18). For the granule secretion, we have recently demonstrated the direct involvement of PKC (19). We have established a semi-intact secretion assay (19, 20) where the secretion does not occur upon stimulation without adding exogenous cytosol, indicating the existence of cytosolic essential factors. We purified an essential...
RESULTS

Establishment of an Assay Analyzing the Ca^{2+}-induced Aggregation of Permeabilized Platelets—We have established an aggregation assay with platelets permeabilized by SLO, which has been shown to form pores of ~30 nm in diameter in the membrane (27). In this method, we permeabilized only the plasma membrane judging from observations that the intracellular membrane structures of the platelets appeared to be intact morphologically (30) and that vWF stored in α-granules did not leak out (19, 20). Because the condition used here with 0.6 μg/ml SLO induced leakage of 80% cytosolic lactate dehydrogenase from platelets (19, 20), it was presumed that most of ATP and cytosol were also lost by diffusion through the pores in the plasma membrane. Therefore, we exogenously added ATP and cytosol in the assay to reconstitute the aggregation. We also added 0.4 mg/ml fibrinogen in our assay, which would bridge the activated integrin α_{IIb}β_{3} on both sides of platelets to be aggregated (2, 3). Because calcium ionophore has been demonstrated to induce platelet aggregation (31, 32), we used calcium ions at a calculated concentration at 200 μM (25) as a stimulus.

We first examined morphologically whether aggregates of permeabilized platelets were indeed generated in the assay. After confirming the Ca^{2+}-induced aggregation by the light-transmission aggregometer (data not shown), the samples were subjected to observation with a phase-contrast microscope. A typical set of photographs showed that many platelet aggregates formed upon stimulation with 200 μM Ca^{2+}, whereas the permeabilized platelets incubated with 20 nM Ca^{2+} remained unaggregated (Fig. 1A). The quantification of the platelets in the images revealed that unaggregated platelets were reduced upon Ca^{2+} stimulation (Fig. 1B) and that the aggregates consisting of >10 platelets were drastically increased (Fig. 1C). Thus, the formation of the aggregates in the assay was confirmed morphologically.

We next examined the effects of Ca^{2+} concentrations on the platelet aggregation. Although Ca^{2+} at 20 and 200 nM did not induce the platelet aggregation, Ca^{2+} at 2–200 μM efficiently induced the aggregation (Fig. 2). Because it has been shown that [Ca^{2+}] in resting platelet cytosol is around 10 nM and that it increases to 1–10 μM upon platelet activation (33), the Ca^{2+} sensitivity of the aggregation in the assay using permeabilized platelets was similar to physiological conditions.

Aggregation of Permeabilized Platelets Was Mediated by the Integrin—It has been well known that platelet aggregation is mediated by activated integrin α_{IIb}β_{3} (2, 3). We examined whether it was the case for the aggregation of permeabilized platelets. RGD is the integrin-binding sequence (34), which is present in both fibrinogen and vWF, and the aggregation of intact platelets has been shown to be inhibited by the addition of the RGD-containing peptide (35, 36). As shown in Fig. 3, the RGDs peptide inhibited the Ca^{2+}-induced aggregation of permeabilized platelets, whereas the control RGES peptide did not. Furthermore, when fibrinogen was omitted from the assay, the aggregation was also inhibited (data not shown). Taken together, the aggregation in the assay was mediated by the integrin.

Platelet Aggregation Was Cytosol-dependent—ATP and cytosol in the permeabilized platelets would be lost by diffusion through the pores. Without the addition of ATP, the permeabilized platelets did not aggregate upon the Ca^{2+} stimulation (data not shown), indicating that ATP is essential for the aggregation. When the cytosol was not added exogenously into the assay, the permeabilized platelets did not aggregate upon the Ca^{2+} stimulation in the condition where ATP and fibrino-
gen were sufficiently supplemented (Fig. 4A). On the other hand, the aggregation was reconstituted by the addition of platelet cytosol in a concentration-dependent manner (Fig. 4A).

These results indicated the existence of cytosolic essential factor(s). We next tested rat brain cytosol for the reconstitution of the aggregation. The rat brain cytosol also supported the aggregation as efficiently as the human platelet cytosol (Fig. 4B).

Fig. 1. Morphological examination of the Ca^{2+}-induced aggregation of permeabilized platelets. A, the permeabilized platelets were incubated with 20 nM Ca^{2+} (Ca^{2+} stimulation (-)) or 200 μM Ca^{2+} (Ca^{2+} stimulation (+)) at 37°C for 20 min and examined by phase-contrast microscopy (×1000) as described under “Experimental Procedures.” An arrowhead and an arrow indicate an unaggregated platelet and an aggregate consisting of >10 platelets, respectively. B and C, 20 individual fields of the sample after the incubation were randomly selected, and numbers of unaggregated platelets (B) and aggregates consisting of >10 platelets (C) were counted. The data shown are expressed as means ± S.E. of four independent experiments.

Fig. 2. The Ca^{2+} concentration-dependent aggregation of the permeabilized platelets. Permeabilized platelets were first incubated for 30 min at 4°C with 2 mg of proteins/ml rat brain cytosol followed by stimulation with indicated concentrations of Ca^{2+} (25) as described under “Experimental Procedures.” The data shown are the representative of four independent experiments with similar results.

Fig. 3. The Ca^{2+}-induced aggregation was inhibited by the RGDS peptide, an integrin α_{IIb}β_{III} inhibitor. Permeabilized platelets were first incubated for 30 min at 4°C with 2 mg of proteins/ml rat brain cytosol in the absence or presence of 1 μM RGDS-peptide or RGES-peptide, and the Ca^{2+}-induced aggregation was analyzed as described under “Experimental Procedures.” The data shown are the representative of four independent experiments with similar results.

Fig. 4. The Ca^{2+}-induced aggregation was cytosol-dependent. A, the permeabilized platelets were first incubated for 15 min at 4°C with indicated concentrations of platelet cytosol, and the Ca^{2+}-induced aggregation was analyzed as described under “Experimental Procedures.” B, the permeabilized platelets were first incubated for 15 min at 4°C with rat brain cytosol or human platelet cytosol at 1.5 mg of proteins/ml, and the Ca^{2+}-induced aggregation was analyzed as described under “Experimental Procedures.” The data shown are the representative of four independent experiments with similar results.
Western blot with the anti-PKC/H9251 in platelets, whereas PKC/GF109203X, and the Ca\(^{2+}\) inhibitor. These results indicated that cytosolic essential factor(s) were expressed ubiquitously.

**Involvement of PKC in the Regulation of Platelet Aggregation**—The cytosol dependence of the aggregation indicates that some cytosolic factors are required for the platelet aggregation. Although the identity of these factors is unknown, one important factor could be cPKC. As shown previously with intact platelets (37), GF109203X, an inhibitor of cPKCs, also affected the Ca\(^{2+}\)-induced aggregation in our semi-intact assay in a concentration-dependent manner (Fig. 5).

To examine the involvement of PKC directly, we first prepared PKC-depleted platelet cytosol. As shown in Fig. 6A, PKC\(\alpha\) was completely depleted from the platelet cytosol with the anti-PKC\(\alpha\) antibody-coated beads while PKC\(\gamma\) stayed in the cytosol after the same procedure with control IgG-coated beads. Among other cPKCs, PKC\(\beta_1\) and PKC\(\beta_\text{II}\) were detected in platelets, whereas PKC\(\gamma\), a neuronal specific cPKC, was not (data not shown). By the immunodepletion, PKC\(\beta\) was also completely depleted because of the cross-reactivity of the antibody (Fig. 6A). When we used lower amounts of the anti-PKC\(\alpha\) antibody for the immunodepletion, PKC\(\beta\) was completely depleted while PKC\(\alpha\) still remained in the cytosol (data not shown), suggesting that platelet cytosol contained more PKC\(\alpha\) than PKC\(\beta\). As expected, although PKC\(\delta\) and PKC\(\theta\), both of which are classified as novel PKCs, were detected in platelets, they were not affected by the immunodepletion procedure either with anti-PKC\(\alpha\) antibody or control IgG (Fig. 6A).

The PKC-depleted cytosol lost the aggregation activity, whereas the Ca\(^{2+}\)-induced platelet aggregation was efficiently reconstituted with the cytosol treated with the control IgG (Fig. 6C). When PKC\(\alpha\) purified from rat brain (Fig. 6B) (19) was supplemented to the PKC-depleted cytosol, the aggregation activity was recovered (Fig. 6C), indicating that cPKC, possibly PKC\(\alpha\), is an essential cytosolic factor for the platelet aggregation. We next examined whether PKC\(\alpha\) is a sufficient cytosolic factor for the aggregation. As shown in Fig. 7, purified PKC\(\alpha\) (50 nM) alone was not sufficient to support the Ca\(^{2+}\)-induced platelet aggregation. On the other hand, platelet cytosol at 0.6 mg of proteins/ml, which contained 15 nM PKC\(\alpha\) determined by Western blot with the anti-PKC\(\alpha\) antibody using purified PKC\(\alpha\) as a control (data not shown), efficiently induced platelet aggregation (Fig. 7). Thus, PKC\(\alpha\) is not a sufficient cytosolic factor for platelet aggregation. Furthermore, the addition of purified PKC\(\alpha\) to the low concentration of cytosol (0.6 mg of proteins/ml) strongly enhanced the platelet aggregation, suggesting that PKC\(\alpha\) is a limiting factor for the Ca\(^{2+}\)-induced platelet aggregation. Thus, PKC\(\alpha\) is an essential but not sufficient cytosolic factor for the Ca\(^{2+}\)-induced platelet aggregation.

**DISCUSSION**

Here we established an aggregation assay system using permeabilized platelets, and using this assay, we first directly demonstrated that PKC is an essential but not sufficient factor in the cytosol for platelet aggregation.

Because platelets lack the protein-producing activity, it is difficult to apply molecular biology for investigating the molecular mechanism of aggregation and granule secretion inside activated platelets. Therefore, the research in these fields has been performed mainly pharmacologically. To overcome this difficulty, much effort has been made to establish semi-intact assay systems using permeabilized platelets. In the research of platelet granule secretion, several semi-intact assays have been established (4–8, 19, 20). However, for platelet aggregation, only a few semi-intact aggregation assays have been established (8), and as far as we know, no stable assays with cytosol dependence have been established.
in vitro was induced without adding exogenous cytosol (23). Here, we since the time course, the Ca\(^{2+}\) lets were first incubated for 15 min at 4°C in the Ca\(^{2+}\)-induced aggregation was analyzed as described under “Experimental Procedures.” It is noted that 1 mg of proteins/ml platelet cytosol contains 55 nM PKC. The data shown are the representative of four independent experiments with similar results.

We have previously established an aggregation assay system with SLO-permeabilized platelets and demonstrated that small GTPase Rho plays an important role in thrombin-induced aggregation (23). However, the assay did not demonstrate cytosol dependence since a low concentration of SLO (0.1 μg/ml) was used for the permeabilization and the aggregation was induced without adding exogenous cytosol (23). Here, we have established another semi-intact aggregation assay by modifying the previous one (23). The aggregation of the permeabilized platelets in our in vitro assay appears physiological since the time course, the Ca\(^{2+}\) sensitivity and the involvement of the integrin are similar to those of intact platelets. Since the cytosol was extensively depleted from the permeabilized platelets in our semi-intact aggregation assay using a higher concentration of SLO than that used previously (23), permeabilized platelets did not aggregate in response to calcium stimulation without adding exogenous cytosol. This cytosol dependence would widen the application of the assay to investigations aimed at elucidating these mechanisms.

The cytosol dependence also indicated the existence of essential cytosolic factor(s) for aggregation. A cytosolic protein PKCα has been shown to play an important role in platelet aggregation by pharmacological experiments using cell-permeable small compounds of inhibitors and stimulators such as phorbol esters (16, 17). However, the results obtained from such experiments appear somewhat indirect because the specificity of inhibitors is not absolutely strict and important signaling molecules containing the phorbol ester-binding C1 domain other than PKCα have been recently identified such as Ras-guanyl nucleotide-releasing protein (11, 12) and chimerin (13, 14). Munc13-1 present in the presynapse also contains the C1 domain (38), and it has very recently been demonstrated that the effect of phorbol ester in the neurotransmitter release is through Munc13-1 (39). Thus, at the moment, it is ambiguous whether phorbol esters exert their functions through PKCα or other non-PKC-signaling molecules. Therefore, re-evaluation and direct demonstration are required in various cell functions where PKCs have been suggested to be involved (15, 43).

Using the semi-intact aggregation assay, we have directly demonstrated the involvement of PKCα in the Ca\(^{2+}\)-induced platelet aggregation. First, an inhibitor of conventional PKC affected the aggregation. Second, immunodepletion of PKCα and PKCβ from the cytosol abolished the Ca\(^{2+}\)-induced aggregation. Third, the aggregation-supporting activity of PKCα/β-depleted cytosol was rescued by supplementation of purified PKCα. Supplementation of PKCα alone to the PKCα/β-depleted cytosol was enough to reconstitute the aggregation, suggesting that PKCα but not PKCβ is the essential factor or otherwise that the activity of cPKC, namely PKCα or PKCβ, is essential. Because PKCα and PKCβ show similar substrate specificity in vivo (40), we cannot exclude the possibility that added PKCα covered the lack of PKCβ activity in the assay. Although cPKC is an essential factor for the aggregation, it is not a sufficient cytosolic factor since the addition of purified PKCα alone without exogenous cytosol did not support the aggregation. Furthermore, platelet cytosol containing less PKC supported the aggregation efficiently, and purified PKCα strongly enhanced the aggregation in the presence of low concentration of cytosol, suggesting that PKCα is a limiting factor in the cytosol for the aggregation and that additional factors besides PKC would also be required for the aggregation.

PKCα and PKCβ are known to be activated by Ca\(^{2+}\), diacylglycerol, and phosphatidylinerine (9, 10). Although we did not add these stimulators besides Ca\(^{2+}\), the purified PKCα added to the assay was indeed active since the purified PKCα phosphorylated a PKC substrate efficiently in the similar assay condition used here (19). We speculate that the components inside the platelets, possibly including phosphatidylinerine, help support the activity of PKCα (19). Furthermore, because PKC increases the intracellular Ca\(^{2+}\) concentration by modulating Ca\(^{2+}\) channels in the plasma membrane in neurons (41, 42), it remains unclear whether PKC acts upstream and/or downstream of increased Ca\(^{2+}\). Because we used Ca\(^{2+}\) as a stimulus, we could safely say that PKCα plays an important role at least at the downstream of increased Ca\(^{2+}\). Further investigation is required for elucidation of how PKCα activates the integrin αIIbβ\(3\) and induces platelet aggregation. The assay established here will be a powerful tool for future experiments aimed at elucidating these mechanisms.

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REFERENCES

1. Shattil, S. J., Kashiwagi, H., and Pampori, N. (1998) Blood 91, 2645–2657
2. Authi, K. S., Rao, G. H., Evenden, B. J., and Crawford, N. (1988) Biochem. J. 255, 885–893
3. Flausenhoff, R., Furie, B., and Furie, B. C. (1999) J. Cell. Physiol. 179, 1–10
4. Inui, T., and Haslam, R. J. (1997) Biochem. J. 328, 13–21
5. Lemons, P. P., Chen, D., Bernstein, A. M., Bennett, M. K., and Whiteheart, S. W. (1997) Blood 90, 1490–1500
6. Hirvonen, A., Renath, J., Litjens, P. E., van Willigen, G., and Akkerman, J. W. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 1651–1660
7. Nishizuka, Y. (1992) Science 258, 697–614
8. Newton, A. C., and Johnson, J. E. (1998) Biochim. Biophys. Acta 1376, 158–172
9. Ebihara, J. O., Botorti, D. A., Chan, E. Y., Stang, S. L., Dunn, R. J., and Stone, J. C. (1998) Science 280, 1082–1086
10. Tognon, C. E., Kirk, H. E., Faumure, L. A., Whitehead, I. P., Der, C. J., and Kay, R. J. (1998) Mol. Cell. Biol. 18, 6995–7008
11. Hall, C., Monfried, C., Smith, P., Lim, H. H., Kozma, R., Ahmed, S., Vassilakopoulos, V., Lent, P., and Lim, L. (1990) J. Mol. Biol. 211, 11–16
12. Wang, H., and Kazanietz, M. G. (2002) J. Biol. Chem. 277, 4451–4450
13. Kazanietz, M. G., Caloca, M. J., Eroles, P., Fujii, T., Garcia-Bermejo, M. L., Beilby, M., and Wang, H. (2000) Biochem. Pharmacol. 60, 1417–1424
14. Kashiwagi, H., Takai, Y., Sawamura, M., Hoshijima, M., Fujikura, T., and Nishizuka, Y. (1983) J. Biol. Chem. 258, 6701–6704
15. Siess, W., and Lapetina, E. G. (1988) Biochem. J. 255, 309–318
18. Gerrard, J. M., Beattie, L. L., Park, J., Israels, S. J., McNicol, A., Lint, D., and Cragoe, E. J., Jr. (1989) **Blood** **74**, 2405–2413

19. Yoshioka, A., Shirakawa, R., Nishioka, H., Tabuchi, A., Higashi, T., Ozaki, H., Yamamoto, A., Kita, T., and Horuchi, H. (2001) **J. Biol. Chem.** **276**, 39379–39384

20. Shirakawa, R., Yoshioka, A., Horuchi, H., Nishioka, H., Tabuchi, A., and Kita, T. (2000) **J. Biol. Chem.** **275**, 33844–33849

21. Bradford, M. M. (1976) **Anal. Biochem.** **72**, 248–254

22. Laemmli, U. K. (1970) **Nature** **227**, 680–685

23. Nishioka, H., Horuchi, H., Tabuchi, A., Yoshioka, A., Shirakawa, R., and Kita, T. (2001) **Biochem. Biophys. Res. Commun.** **280**, 970–975

24. Phillips, D. R., and Agin, P. P. (1977) **J. Clin. Invest.** **60**, 535–545

25. Fabiato, A., and Fabiato, F. (1979) **J. Physiol. (Paris)** **75**, 463–505

26. Ullrich, O., Horuchi, H., Bucci, C., and Zerial, M. (1994) **Nature** **368**, 157–160

27. Palmer, M., Harris, R., Freytag, C., Keboe, M., Tranum-Jensen, J., and Bhakdi, S. (1988) **EMBO J.** **17**, 1598–1605

28. Kinlough-Rathbone, R. L., Perry, D. W., Rand, M. L., and Packham, M. A. (1999) **Thromb. Res.** **95**, 315–323

29. Santos, M. T., Moscardo, A., Valles, J., Martinez, M., Pinon, M., Aznar, J., Broekman, M. J., and Marcus, A. J. (2000) **Circulation** **102**, 1924–1930

30. Yoshio, A., Horuchi, H., Shirakawa, R., Nishioka, H., Tabuchi, A., Higashi, T., Yamamoto, A., and Kita, T. (2001) **Ann. N. Y. Acad. Sci.** **947**, 403–406

31. Girolami, A., Fabris, F., Marco, L., and Peruffo, R. (1976) **Acta Haematol.** **56**, 151–159

32. Botteccia, D., Fantin, G., group, F., and Nassuato, G. (1976) **Haemostasis** **5**, 176–188

33. Knight, D. E., Hallam, T. J., and Scrutton, M. C. (1982) **Nature** **296**, 30–33

34. Pierschbacher, M. D., and Ruoslahti, E. (1984) **Nature** **309**, 30–33

35. Leffkovits, J., Plow, E. F., and Topol, E. J. (1995) **N. Engl. J. Med.** **332**, 1553–1559

36. Basani, R. B., D'Andrea, G., Mitra, N., Vilaire, G., Richberg, M., Kowalska, M. A., Bennett, J. S., and Ponec, M. (2001) **J. Biol. Chem.** **276**, 13975–13981

37. Toullec, D., Pianetti, P., Cotes, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boisson, P., Boursier, E., Loriole, F., Duhamel, L., Charon, D., and Kirilovsky, J. (1991) **J. Biol. Chem.** **266**, 15771–15781

38. Brose, N., Hofmann, K., Hata, Y., and Sudhof, T. C. (1995) **J. Biol. Chem.** **270**, 25273–25280

39. Rhee, J. S., Beta, A., Pyott, S., Reim, K., Varoqueaux, F., Augustin, I., Hesse, D., Sudhof, T. C., Takahashi, M., Rosenmund, C., and Brose, N. (2002) **Cell** **108**, 121–133

40. Hofmann, J. (1997) **FASEB J.** **11**, 649–669

41. Swartz, K. J. (1993) **Neuron** **11**, 305–320

42. Stea, A., Soong, T. W., and Snutch, T. P. (1995) **Neuron** **15**, 929–940

43. Brose, N., and Rosenmund, C. (2000) **J. Cell. Sci.** **113**, 1399–1411
Direct Demonstration of Involvement of Protein Kinase Cα in the Ca^{2+}-induced Platelet Aggregation

Arata Tabuchi, Akira Yoshioka, Tomohito Higashi, Ryutaro Shirakawa, Hiroaki Nishioka, Toru Kita and Hisanori Horiuchi

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