The Mechanism of Arachidonic Acid Release in Collagen-activated Human Platelets

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The mechanism of arachidonic acid (AA) release in collagen-activated human platelets was studied. An arachidonic acid metabolite, thromboxane B₂ (TXB₂), was formed in parallel with the formation of phosphatidic acid (PA) without formation of lysophosphatidic acid (lysoPA) or lysophosphatidylinositol (lysoPI) in the absence of extracellular Ca²⁺, suggesting that AA was released from PI via a PI-specific phospholipase C (PI-PLC)/dialyglycerol (DG) lipase/monoacylglycerol (MG) lipase pathway under the cytosolic low Ca²⁺ concentrations. Moreover, soluble DG lipase and MG lipase could hydrolyze the substrates at basal cytosolic free Ca²⁺ concentrations. Subsequently, the relationship of cytosolic free Ca²⁺ concentrations and formation of AA metabolites was analyzed using Ca²⁺ ionophore, A23187. Collagen was able to induce a release of small amounts of AA under basal cytosolic Ca²⁺ conditions. However, a release of large amounts of AA was induced by phospholipase A₂ activated by both collagen-receptor occupancy and elevated Ca²⁺ levels. A TXA₂ mimetic agonist, STA₂ induced all the responses except for AA release. From these results, the mechanism of AA release and signal transduction in collagen-activated human platelets is discussed.

When human platelets are stimulated with various agonists such as thrombin or collagen, the platelets are aggregated via several biochemical events. The earliest event during the stimulation of human platelets is acceleration of phosphoinositide (PI) metabolism. The initial reaction of the metabolism is the hydrolysis of phosphoinositides by PI-specific phospholipase C (PI-PLC). Inositol 1,4,5-trisphosphate (IP₃), one of the reaction products, mobilizes ionized calcium from intracellular stores as a second messenger. IP₃ triggers Ca²⁺ mobilization in platelets activated with thrombin. Stimulation with collagen also elicited elevation of cytosolic free Ca²⁺ concentrations. However, in the presence of cyclooxygenase inhibitors such as indomethacin or aspirin, the rise in cytosolic free Ca²⁺ concentrations in collagen-activated platelets was suppressed. This suggests that collagen itself cannot evoke an increase in cytosolic free Ca²⁺ and that arachidonic acid (AA) metabolites are involved in the elevation of cytosolic free Ca²⁺. It was previously demonstrated that several nanomolar thromboxane A₂ (TXA₂) directly caused Ca²⁺ mobilization without further activation of PI-PLC during activation with collagen. Hence, it is likely that TXA₂ is important for elevation of cytosolic free Ca²⁺ in collagen-activated human platelets. The rate-limiting step in the process of TXA₂ formation is the release of AA from membrane phospholipids. PI was hydrolyzed to supply free AA in collagen-activated human platelets in the absence of extracellular Ca²⁺ and other phospholipids were degraded in the presence of extracellular Ca²⁺. In the absence of extracellular Ca²⁺, cytosolic free Ca²⁺ concentrations were elevated from 50 nm to about 100 nm, while cytosolic free Ca²⁺ concentration was elevated to about 300 nm in the presence of extracellular Ca²⁺. This suggests that concentrations of cytosolic free Ca²⁺ determine the degradation of phospholipid classes which supply AA to cytosol. In this paper, we attempted to identify the pathway(s) responsible for AA release at the early stage of stimulation and find the mechanism of AA release in connection with cytosolic free Ca²⁺ concentrations during activation of human platelets with collagen.

Materials and Methods

Materials. Phosphatidylethanolamine plasmalogen (bovine brain) was purchased from Serdary Research Laboratory, Aquaasol-2, [1-¹⁴C]stearic acid, [1-¹⁴C]arachidonic acid, [¹⁴C]arachidonic acid, and TXB₂ radioimmunossay kit were from Du Pont—New England Nuclear (Boston, MA, U.S.A.). Carrier-free [³²P]Pip was from the Japan Radioisotope Association (Tokyo, Japan). Leupeptin was from the Peptide Institute (Osaka, Japan). Hepatitis B-globulin and fura-2 acetoxyethyl esters were from Dojindo Laboratories (Kumamoto, Japan). Collagen was from Hormon-Chemie (Munchen, Germany). The TXA₂ antagonist, ONO-3708 (9,11-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epi-TXA₂) and the TXA₂ analogue, STA₂ (9,11-epithio-1,12-methano-TXA₂) were gifts from Dr. A. Kawasaki of the Research and Development Division, Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Phospholipase C (grade 1, from Bacillus cereus) was purchased from Boehringer Mannheim. All other chemicals were of reagent grade and obtained from commercial sources.

Preparation of human platelets. Blood was drawn from normal human volunteers into a 13% solution of 102 mM sodium citrate (tribasic), 15.6 mM citric acid, 17.7 mM sodium phosphate ( dibasic), and 128.8 mM glucose as an anticoagulant. Platelet-rich plasma was obtained by centrifugation at 1100 x g for 5 min, followed by a 4-fold concentration by centrifugation at 3800 x g for 5 min. The concentrated platelet-rich plasma was then centrifuged at 100 x g for 10 min to remove red cells. For loading of fura-2, the concentrated platelet rich plasma was incubated with 3 μM fura-2 acetoxyethyl ester at 37°C for 60 min. For [³²P]Pip labeling, the concentrated platelet-rich plasma was incubated with [³²P]Pip (2 mCi/20 μl).

Abbreviations: AA, arachidonic acid; TXA₂ (B₂); thromboxane A₂ (B₂); PI, phosphoinositide(s); PLC, phospholipase C; PLA₂, phospholipase A₂; DG, dialyglycerol; MG, monoacylglycerol; PA, phosphatidic acid; EGTA, [ethylene bis(oxyethylenemirito)]tetraacetie acid; PMSF, phenylmethylsulfonyl fluoride; MES, 2-(N-morpholino) ethanesulfonic acid.

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at 37°C for 60 min. For [14C]arachidonic acid labeling, the concentrated platelet-rich plasma was incubated with [14C]arachidonic acid (0.75 μCi/45 μl) at 37°C for 60 min. After these, platelets were washed twice by centrifugation at 1500 g for 10 min with Tris-citrate-bicarbonate buffer, pH 6.5 containing 2 mM EDTA. Washed platelets were suspended in Tris-citrate-bicarbonate buffer, pH 6.9 without EDTA.

Measurement of cytosolic free Ca2+ concentrations. Fura-2-loaded human platelets (2 x 10^9/ml) prepared as described above were incubated fo 1-3 min at 37°C with or without ONO-3708 (0.1 μM) before activation. They were then activated with collagen (10 μg/ml) or various concentrations of A23187 with gentle stirring. The ratio of fluorescence intensities (500 nm, emission) at two excitation wavelengths of 340 and 380 nm was continuously monitored with a Ca2+ analyzer (CAF-100, Japan Spectroscopic Co., Ltd.). The cytosolic free Ca2+ concentration was calculated by the use of a dissociation constant for the fura-2-Ca2+ complex of 224 nm.10

Analysis of phospholipid metabolism. 3H-label-laughed platelet suspension (2 x 10^9/ml) was incubated with 5 mM EGTA for 3 min at 37°C and activated with collagen (10 μg/ml). Aliquots (1 ml) were removed at various times and mixed with 3.6 ml of chloroform–methanol–conc. HCl (100: 200: 2, v/v). The phases were separated by adding 1.2 ml of chloroform and 1.2 ml of 2 M KCl/0.1 M EDTA. The lower organic layer was removed and the aqueous layer was washed twice with 2.5 ml of chloroform. The combined organic layer was dried under N2 flow. The lipids were separated on silica gel G plates (Merck) using chloroform–methanol–acetic acid (4: NH2OH (45: 35: 10, v/v)). After drying in vacuo for 1 h at room temperature, the plates were redeveloped with the same solvent in the same direction. The lipids were made visible by autoradiography, scraped off from the plate and counted by liquid scintillation in Aquasol–2–water methanol (83:12.5:1.5, v/v).

Analysis of arachidonic acid metabolism. [14C]Arachidonic acid–labeled platelet suspension (2 x 10^9/ml; 7.5 ml) was incubated with 5 mM CaCl2 and 0.1 μM ONO-3708 for 3 min at 37°C. Then, collagen (10 μg/ml) or vehicle was added into platelet suspension, followed by stimulation with various concentrations of A23187. After 3 min, the reaction was stopped by adding 15 ml of chloroform–methanol (1:2, v/v) containing 0.01% BHT, 0.1 mM 2-mercaptoethanol, and 7.5 ml of 2 M KCl/0.1 M EDTA. After this, it was shaken for 10 min, 15 ml of chloroform was added, followed by shaking again for 10 min. After centrifugation, the upper aqueous layer was separated and washed twice with chloroform. The combined organic layer was dried under N2 flow. The lipids were separated on silica gel G plates (Merck) using chloroform–methanol–acetic acid water (100: 50: 100: 4, v/v) as mobile phase. The AA metabolites and AA-containing lipids were made visible by autoradiography and counted as described above. The sum of radioactivities corresponding to 2-hydroxyoctadecatrienoic acid, hydroxyicosatetraenoic acid, and TXB2 was counted as production of AA metabolites. To examine the formation of AA metabolites in ST2A-stimulated human platelets, various concentrations of ST2A were added to a platelet suspension (2 x 10^9/ml; 7.5 ml). After 30 sec or the indicated time, AA metabolites produced were measured as described above. For measurement of TXB2, a TXB2 radioimmunoassay kit was used.

Preparation of crude DG lipase and MG lipase sample. Washed human platelets prepared as described above without labeling or labeling were suspended in the lysis buffer (20 mM Tris-HCl (pH 7.4), 20 mM EGTA, 0.5 mM leupeptin, 1 mM dithiothreitol) and sonicated twice with a Branson sonicator model 250 at setting 3 for 30 s. The sonicated platelets were centrifuged at 200,000 g for 1 h and then separated into a cytosolic fraction and membrane fraction. The resultant membrane fraction was solubilized by solubilizing buffer (20 mM Tris–HCl, pH 7.4, 5 mM EDTA, 0.5 mM PMSF, 1 mM dithiothreitol, 30% glycerol, 2% heptyl-β-D-thioglycolate, 4% N,N',N'-tetramethyl-1,4-phenylenediamine by 0.05 N HCl, 0.1 M H2O2, ethanol for 2 h at 37°C. The generated 2-acetylphosphatidylethanolamine was isolated by thin-layer chromatography using chloroform–methanol–acetone–acetic acid–water (100: 50: 100: 4: 10, v/v). The resultant 2-acetylphosphatidylethanolamine was extracted from scraped TLC powder with chloroform–methanol (2:1, v/v). [14C]Stearylphosphatidylethanolamine was biosynthetically from rat liver microsomes using [14C]stearic acid and 2-acetylsphosphatidylethanolamine as described by Lands and Merkl.11 [14C]Stearyl-diacylglycerol was observed by treating with [14C]stearoylphosphatidylethanolamine with phospholipase C (Bacillus cereus) for 3 h at 30°C. The obtained labeled diacylglycerol was dissolved in ethanol and stored at −20°C to keep the isomerization of 2- to 1-acetyl migration to a minimum. To prepare a labeled substrate for MG lipase assay, 1-stearoyl-2-[14C]arachidonoylphosphatidylcholine was bio-synthesized as described above using 1-stearoyl-2-lysophosphatidylcholine and [14C]-arachidonic acid. The labeled phospholipid was hydrolyzed by phospholipase C as mentioned above. The resultant labeled diacylglycerol was suspended in 1 ml of 0.18 M Tris–HCl buffer (pH 6.5) containing bovine serum albumin (2.27 mg/ml), CaCl2 (18 mM), H3BO3 (18 mM), and Triton X-100 (0.145 mg/ml). The suspension was dispersed by sonication for 1 min twice at 4°C. After sonication, 0.9 ml of pancreatic lipase (Sigma, 0.1 mg/ml) was added to the suspension and incubated for 30 min at 37°C with gentle shaking.12 The reaction was stopped by adding 4 ml of chloroform–methanol (2:1, v/v) and 0.2 ml of conc. HCl. After shaking by hand, phases were separated by centrifugation. The upper phase was washed twice with 2.6 ml chloroform. The lower phases were combined, dried under N2 flow, and dissolved in ethanol. The 2-[14C]arachidonoylmonoacylglycerol obtained was kept at −20°C.

Enzyme assays. DG lipase assay was done as follows. The radiolabeled diacylglycerol (approx. 40,000 cpm = 10 nmol per one assay) was transferred into 80 μl of 50 mM MES NaOH, pH 7.0, followed by vortex mixing, and sonicated twice with a Branson sonicator model 250 at setting 8 for 1 min. The reaction was started by mixing with the enzyme source (20 μl). After incubation for 60 min at 37°C, the reaction was stopped by the addition of 1.5 ml of chloroform–methanol–heptane (125: 140: 100, v/v). Then, stearic acid (20 μg of 25 mM soln.) as carrier and 0.5 ml of K2CO3, KHCO3 (pH 10.0) were added and mixed with a vortex for 10 s. The phase separation was done by centrifugation and samples of the upper layer were used for scintillation counting in 4 ml of Aquasol-2. MG lipase assay was done under the conditions described for DG lipase assay using radiolabeled monoacylglycerol (approx. 10,000 cpm = 10 nmol per assay) and 25 mM arachidonic acid as substrate and carrier, respectively. Various free Ca2+ concentrations were obtained by using Ca2+-EGTA buffers (pH 7.0) containing 2 mM EGTA (final concentration) and the appropriate amount of CaCl2.14

Results
Phospholipid metabolism during activation of human platelets with collagen
The formation of PA, lysOPA, and lysOPl in collagen-activated human platelets was measured in the absence of extracellular Ca2+. PA was increased in a time-dependent manner, but lysOPA and lysOPl were not formed under these conditions (Fig. 1A). The formation of TXB2 increased in parallel with the formation of PA (Fig. 1B).

Effects of Ca2+ on DG lipase and MG lipase activities
We investigated whether MG and DG lipases could be activated under the low Ca2+ condition. These enzymes were solubilized from the microsome fraction of human platelets. About 70–80% of total activities and about 30% of total proteins of the crude membrane fraction were solubilized by 2% heptyl-β-D-thioglycolide (data not shown). Then, effects of Ca2+ concentrations on the solubilized enzymes were measured. As shown in Fig. 2, DG lipase activity was not significantly affected in the presence of various Ca2+ concentrations, but MG lipase activity was slightly affected by Ca2+. The hydrolytic activity of MG lipase was maximal at 10−7–10−8 M Ca2+ concentrations, while higher Ca2+ concentrations showed rather an inhibitory effect.
Fig. 1. Lipid Metabolism during Stimulation of Human Platelets with Collagen in the Absence of Extracellular Ca\(^{2+}\).
A. [\(^{32}\)P]Pi-labeled platelets were stimulated with 10 ng/ml collagen in the presence of 5 mM EGTA. At appropriate intervals, samples were removed and analyzed as described in Materials and Methods. (□), PA; (●), lysoPA; (○), lysoPL.
B. Cold platelets were stimulated as described above. At appropriate intervals, aliquots were removed and TXB\(_2\) were measured as described in Materials and Methods. Data are given as mean ± S.D.

Fig. 2. Effects of Ca\(^{2+}\) on DG Lipase and MG Lipase Activities from Platelet Microsome.
DG lipase or MG lipase activities were measured in the buffer containing various concentrations of Ca\(^{2+}\) and 100 μM of 1-[\(^{14}\)C]arachidonoylglycerol or 2-[\(^{14}\)H]arachidonoylmonoacylglycerol, respectively. [Ca\(^{2+}\)] was adjusted by Ca\(^{2+}\)-EGTA buffers, pH 7.0. The assays were done as described in Materials and Methods. The results are expressed as the means for three separate experiments.

Effects of cytosolic free Ca\(^{2+}\) concentrations on arachidonic acid release from phospholipids
The Ca\(^{2+}\) ionophore, A23187, is a pharmacological tool to evoke changes in cytosolic free Ca\(^{2+}\) concentrations.

The effects of A23187 on cytosolic free Ca\(^{2+}\) concentrations in the presence of extracellular Ca\(^{2+}\) were examined in fura-2-loaded human platelets. This reagent rapidly induced the concentration-dependent elevation in cytosolic free Ca\(^{2+}\) concentrations as shown in Fig. 3A. The dose-dependent curve was obtained (Fig. 3B). Thus, it was possible to control cytosolic free Ca\(^{2+}\) concentrations by varying concentrations of A23187. The cytosolic free Ca\(^{2+}\) concentration was not elevated in the presence of ONO-3708, the TXA\(_2\) receptor antagonist, even by stimulation with collagen (Fig. 4B). Then, we studied the relationship with cytosolic free Ca\(^{2+}\) concentrations and the AA release by changing the cytosolic free Ca\(^{2+}\) level by adding various concentrations of A23187 to collagen-stimulated or unstimulated platelets. The changes of cytosolic free Ca\(^{2+}\) concentrations were independent regardless of collagen stimulation (Fig. 4C, D). Only a very small amount of AA metabolites was formed when collagen-untstimulated platelets were treated with 20 nM A23187 (Fig. 5). Under these conditions, concentrations of cytosolic free Ca\(^{2+}\) were elevated to about 300 nM, but when collagen-stimulated platelets were treated with 20 nM A23187, concentrations of cytosolic free Ca\(^{2+}\) were similarly elevated to about 300 nM and AA metabolites were formed at the same level as in the platelets stimulated with collagen without ONO-3708. In the absence of A23187, much more of the AA metabolites were produced in the collagen-stimulated platelets than in the collagen-untstimulated platelets (Fig. 5).
Fig. 4. The Changes of Cytosolic Free Ca\(^{2+}\) Concentrations of Platelets Stimulated with Collagen and/or A23187 in the Presence or Absence of ONO-3708.

Fura-2 loaded platelet suspensions (2 x 10^5/ml) were stimulated by the reagents indicated and the cytosolic free Ca\(^{2+}\) concentrations were monitored as mentioned in Materials and Methods. ONO, ONO-3708; buf, platelet suspending buffer; col, collagen.

Fig. 5. A23187-Induced Elevation of Cytosolic Free Ca\(^{2+}\) Concentrations and Production of Arachidonic Acid Metabolites in Collagen-stimulated or Unstimulated Platelets in the Presence of ONO-3708.

In the presence of ONO-3708, \(^{[14]}\)C-AA or fura-2 loaded platelet suspension was stimulated with or without collagen in the presence of various concentrations of A23187. Cytosolic free Ca\(^{2+}\) concentrations (A) and arachidonic acid metabolites produced in collagen-stimulated (C) or unstimulated (D) platelets were analyzed as described in Materials and Methods. The levels of AA metabolites produced in collagen (10\(\mu\)g/ml)-stimulated platelets in the absence of ONO-3708 (open column). The data shown are means ± S.D. of triplicate determinations.

Formation of AA metabolites and platelet responses by stimulation with STA₂

It was examined whether AA metabolites were formed by stimulation with STA₂, the TXA₂ analogue. As shown in Fig. 6A, STA₂ (up to 100 nM) could not evoke a significant formation of AA metabolites. However, the other platelet responses (cytosolic free Ca\(^{2+}\) elevation, aggregation, and PLC/Arachidonic acid metabolizers) were fully induced. This induction was saturated by stimulation with 100 nM STA₂ (data not shown). The time courses of the formation of AA metabolite formation, cytosolic free Ca\(^{2+}\) elevation, and platelet aggregation by stimulation with 500 nM STA₂ were examined (Fig. 6B). From the results, we concluded that platelets were fully activated by STA₂ without significant formation of AA metabolites.

Discussion

Many studies have been done to reveal the mechanism of AA release in agonist-stimulated cells. In human platelets, we have reported the mass changes of phospholipid molecular species during activation with collagen and thrombin. Thus, only phosphoinositides were hydrolyzed in collagen-activated human platelets under low cytosolic free Ca\(^{2+}\) conditions, but other phospholipids were degraded under high cytosolic free Ca\(^{2+}\) conditions. From these results, we suppose three possible pathways, as shown in Fig. 7, of AA release from PI in collagen-activated human platelets under low cytosolic free Ca\(^{2+}\) concentrations. The first is the pathway catalyzed by PI-PLC, DG lipase, and MG lipase, the second is by PI-PLC, DG kinase, and PA-PLA₂ and the third is by PI-PLA₂. In this paper, we tried to identify the pathway(s) responsible for AA release from PI under the low Ca\(^{2+}\) concentrations...
which reflect the early stage of activation.

One of AA metabolites, TXB₂, was formed in parallel with the formation of PA without formation of lysoPA and lysoPI in the absence of extracellular Ca²⁺ (Fig. 1). PA is the product of PI-PLC and DG kinase, while lysolipids are those of A₂ type of phospholipases (Fig. 7). Therefore, it is likely that PI-PLC may be activated under these conditions, and AA may be released without activations of PLA₂. This indicates that AA may be released from phosphoinositides via PI-PLC/DG lipase/MG lipase pathway at the early stage of stimulation of platelets with collagen, in which cytosolic free Ca²⁺ concentration was at basal level.

Then, we tried to ascertain whether these enzymes could function under basal Ca²⁺ conditions. PI-PLC isozymes have been known to be activated by G protein(s) or tyrosine-phosphorylation(s) under basal Ca²⁺ conditions. In fact, intact PI-PLC purified from human platelets could hydrolyze PIP₂ under nearly basal Ca²⁺ concentrations without other co-factors. However, the minute Ca²⁺ sensitivities of the enzymes which act at the downstream of this PI-PLC pathway have not been well investigated. Hence, we attempted to solubilize DG lipase and MG lipase activities from human platelet microsomes and demonstrated that these lipases can hydrolyze the substrates under basal cytosolic free Ca²⁺ concentrations (Fig. 2). The prescence of this pathway was reported in various tissues, and its physiological significance has been reported. Nevertheless, the molecular properties remain to be described.

The relationship of cytosolic free Ca²⁺ concentrations and formation of AA metabolites using Ca²⁺ ionophore, A23187, was analyzed. A23187 dose-dependently induced cytosolic free Ca²⁺ elevation (Fig. 3). Thus, cytosolic free Ca²⁺ concentrations were able to be controlled by varying concentrations of this reagent. Since AA is released to some extent by only cytosolic free Ca²⁺ elevation, it is necessary to distinguish collagen receptor dependent AA release or elevated cytosolic free Ca²⁺ dependent AA release. Fortunately, cytosolic free Ca²⁺ elevation in collagen-activated platelets depends on TXA₂. Accordingly, elevation of cytosolic free Ca²⁺ was prevented in the presence of the TXA₂ antagonist ONO-3708 (Fig. 4B). It was possible to control cytosolic free Ca²⁺ concentrations in collagen-stimulated or unstimulated platelets kept at nearly the same level (Fig. 4C, D). The amount corresponding to about one third of AA metabolites formed in collagen-stimulated platelets without ONO-3708 was formed in collagen-stimulated platelets in the presence of ONO-3708, in which cytosolic free Ca²⁺ concentration was at the resting level (Fig. 5). This AA release was independent of Ca²⁺ elevation. Therefore, collagen may evoke AA release under basal Ca²⁺ conditions. This release may be caused via the PI-PLC/DG lipase/MG lipase pathway by consideration of our results. AA metabolites in the collagen-unstimulated platelets were at a very low level and were not increased even by stimulation with 20 nm A23187. Under these conditions, cytosolic free Ca²⁺ concentrations were elevated to about 300 nm. When cytosolic free Ca²⁺ concentration in collagen-stimulated platelets was elevated to about 300 nm by stimulation with 20 nm A23187, abundant release of AA was observed. These results suggest that elevation of cytosolic free Ca²⁺ to about 300 nm without collagen stimulation was insufficient for full AA release. Both collagen binding and cytosolic free Ca²⁺ elevation may be necessary for full AA release. Since AA was released from PC, PE, and PI by PLA₂ under high Ca²⁺ conditions, full PLA₂ activation may be done synergistically by both Ca²⁺ elevation and collagen binding. The molecular mechanism of the stimulatory effect of collagen binding for PLA₂ activation is not clear. Several factors have been proposed to be responsible for...
the PLA₂ activation. Pollock et al.²⁶ demonstrated that protein kinase C was not involved in the stimulatory effect of collagen for AA release.

In conclusion, we have shown that the mechanism of AA release during activation with collagen is composed by three steps as follows (Fig. 8). First, at the early stage of stimulation, a small amount of AA is released from PI via PI-PLC/DG lipase/MG lipase pathway, which can be activated under basal Ca²⁺ conditions. By the subsequent conversion to TXA₂, a small amount of TXA₂ at the nm level can elevate cytosolic free Ca²⁺ concentrations.⁶,⁷ Then PLA₂ is activated by synergistic action of elevated Ca²⁺ level and collagen binding. The activated PLA₂ hydrolyzes a large amount of AA from major phospholipids, which is converted to TXA₂. Finally, a large amount of TXA₂ activates either collagen-stimulated platelets or resting platelets. However, the platelets stimulated with TXA₂ alone are aggregated without producing further AA. This may be the termination process of the cascade responses to collagen stimulation.

References