Plasma Membrane Associated Phospholipase C from Human Platelets: Synergistic Stimulation of Phosphatidylinositol 4,5-Bisphosphate Hydrolysis by Thrombin and Guanosine 5'-*O*-(3-Thiotriphosphate)†

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ABSTRACT: The effects of thrombin and GTPγS on the hydrolysis of phosphoinositides by membrane-associated phospholipase C (PLC) from human platelets were examined with endogenous [^{3}H]inositol-labeled membranes or with lipid vesicles containing either [^{3}H]phosphatidylinositol or [^{3}H]phosphatidylinositol 4,5-bisphosphate. GTPγS (1 μM) or thrombin (1 unit/mL) did not stimulate release of inositol trisphosphate (IP_3), inositol bisphosphate (IP_2), or inositol phosphate (IP) from [^{3}H]inositol-labeled membranes. IP_2 and IP_3, but not IP, from [^{3}H]inositol-labeled membranes were, however, stimulated 3-fold by GTPγS (1 μM) plus thrombin (1 unit/mL). A higher concentration of GTPγS (100 μM) alone also stimulated IP_2 and IP_3, but not IP, release. In the presence of 1 mM calcium, release of IP_2 and IP_3 was increased 6-fold over basal levels; however, formation of IP was not observed. At submicromolar calcium concentration, hydrolysis of exogenous phosphatidylinositol 4,5-bisphosphate (PIP_2) by platelet membrane associated PLC was also markedly enhanced by GTPγS (100 μM) or GTPγS (1 μM) plus thrombin (1 unit/mL). Under identical conditions, exogenous phosphatidylinositol (PI) was not hydrolyzed. The same substrate specificity was observed when the membrane-associated PLC was activated with 1 mM calcium. Thrombin-induced hydrolysis of PIP_2 was inhibited by treatment of the membranes with pertussis toxin or pretreatment of intact platelets with 12-*O*-tetradecanoyl-13-acetate (TPA) prior to preparation of membranes. Pertussis toxin did not inhibit GTPγS (100 μM) or calcium (1 mM) dependent PIP_2 breakdown, while TPA inhibited GTPγS-dependent but not calcium-dependent phospholipase C activity. These data demonstrate that the membrane-associated phospholipase C activity in human platelets is activated synergistically by GTPγS plus thrombin and hydrolyzes PIP_2 but not PI.

Phospholipase C catalyzed hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2)[1] is one of the earliest events in the mechanism of transduction of biological signals across the cell surface. Phospholipase C is activated by a variety of agonists that bind to specific cell surface receptors (Abdel-Latif, 1986; Berridge, 1984; Litosch & Fain, 1986). Recent evidence (Baldassare & Fisher, 1986; Haslam & Davidson, 1984; Litosch et al., 1985; Magnaldo et al., 1987; Rebecchi & Rosen, 1987; Straub & Gershengorn, 1986; Uhing et al., 1986; Wallace & Fain, 1985) suggests that the coupling of membrane receptors to phospholipase C is mediated by GTP binding regulatory proteins.

In most cells (Hofmann & Majerus, 1982; Irvine et al., 1979; Ryu et al., 1986), including platelets (Low et al., 1986; Siess & Lapetina, 1983), phosphoinositide-specific phospholipase C is predominantly soluble. In vitro, at high (millimolar) calcium concentrations, the soluble phospholipase C degrades both phosphtidylinositol (PI) and PIP_2 (Hofmann & Majerus, 1982; Irvine et al., 1979). In the presence of physiological calcium and magnesium concentrations, both substrates are poorly hydrolyzed (Baldassare et al., 1988; Irvine et al., 1979). Under these physiological conditions, addition of GTP or GTPγS activates phospholipase C from human platelets to specifically hydrolyze PIP_2 (Baldassare & Fisher, 1986b; Deckmyn et al., 1986). These data suggest that this enzyme may participate in signal transduction in platelets.

Utilizing [^{3}H]inositol-labeled platelet membranes, we previously showed that thrombin and GTPγS synergistically stimulated inositol phosphate formation and that this reaction required calcium (Baldassare & Fisher, 1986b). Similar results have been reported by Hrbolich et al. (1987) and O'Rourke et al. (1987). In contrast, Rock and Jackowski (1987), utilizing platelet membranes and exogenous PIP_2, found that thrombin or GTPγS alone maximally stimulated inositol phosphate formation and that this reaction did not require calcium. Banno and Nozawa (1987) have recently suggested that human platelets contain a unique membrane-associated phospholipase C, which requires detergent for extraction from the membrane. The role of this enzyme in receptor-coupled PIP_2 hydrolysis has not been characterized. We have, therefore, investigated the substrate specificity and regulation by thrombin and GTPγS of the phospholipase C activity in isolated platelet plasma membranes.

Experimental Procedures

Materials. *myo*-[2-^{3}H]Inositol (10-20 Ci/mmol), L-3-phosphatidyl[2-^{3}H]inositol, and phosphatidyl[2-^{3}H]inositol 4,5-bisphosphate were purchased from New England Nuclear (Boston, MA). Phosphatidylethanolamine and phosphatidylinositol were purchased from Avanti Polar Lipids, Inc.

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[1] Abbreviations: PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP, phosphatidylinositol 4-phosphate; PI, phosphatidylinositol; IP, inositol 1-phosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; GTPγS, guanosine 5'-*O*-(3-thiotriphosphate); Gpp(NH)p, guanyl-5'-yl 8-imidodiphosphate; GDPβS, guanosine 5'-*O*-(2-thiodiphosphate); SUV, small unilamellar vesicle(s).

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(Birmingham, AL), and checked for purity on Silica Gel 60 plates (Merck) (Jolles et al., 1981). GTP, GTPγS, GDPβS, and ATP were obtained from Boehringer Mannheim. Pertussis toxin was purchased from List Biological Laboratories (Cambell, CA). PIP_2 was purchased from Sigma and further purified by chromatography on neomycin-linked glass beads as described by Schacht (1978). The purity of the phosphoinositides was checked by TLC (Schacht, 1978). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of [^{3}H]Inositol-Labeled Platelets. Blood was drawn from healthy volunteers into acid/citrate/dextrose (5 mL/60 mL of blood) and incubated with aspirin (0.5 mM) for 15 min at 37 °C. Platelets for 4 units of blood were isolated by differential centrifugation (Timmons & Hawiger, 1978) and incubated for 24–48 h at room temperature in 30 mL of inositol-free Higuchi media with 1 mCi of [^{3}H]inositol. The labeling was carried out in plastic bags used for long-term platelet storage. After incubation, the [^{3}H]inositol-labeled platelets were gel filtered through Sepharose 2B-CL as described previously (Baldassare & Fisher, 1986b).

Preparation of Platelet Membranes. Platelets were lysed by sonication (Sonic 300 Dismembrator, Artek Systems Corp., Farmingdale, NY) at 90 W on ice for 4 × 15 s in Tris-HCl buffer, pH 7.5, with 1 mM EGTA, 0.1 mM dithiothreitol, and leupeptin (100 ng/mL) (buffer A). The platelet sonicate was centrifuged at 100000*g* for 2 h at 4 °C. The 100000*g* pellet was resuspended in buffer A containing 0.25 M sucrose, layered onto 25% sucrose, and spun at 100000*g* at 4 °C for approximately 18 h. Membranes were collected from the 25% sucrose/buffer interface, diluted approximately 3-fold with buffer A, and centrifuged at 100000*g* at 4 °C for 1 h. The membrane pellet was then resuspended in buffer A with 2 M KCl, incubated for 0.5 h at 4 °C, and centrifuged 1 h at 100000*g* at 4 °C. The final platelet membrane pellet was resuspended at 10–15 mg of protein/mL in buffer A.

In those experiments with membranes from PMA-treated platelets, gel-filtered platelets were incubated with PMA (100 ng/mL) for 5 min and membranes prepared as described above.

Phosphoinositide Hydrolysis. [^{3}H]Inositol-labeled membranes (final concentration 100–300 μg of protein) were incubated in a total volume of 200 μL at 37 °C in 100 mM Tris–maleate buffer containing 2.5 mM Mg^{2+} plus 80 mM KCl at the indicated pH. Free Ca^{2+} concentrations were adjusted with Ca^{2+}/EGTA (2 mM EGTA) as described by Raaflaub (1960). After 10 min, the reaction was terminated by the addition of 2 mL of chloroform/methanol/12 N HCl (200:100:0.75 by volume). The [^{3}H]inositol phosphates were extracted and analyzed on 0.5-mL columns of AG 1×8 anion-exchange resin (formate form) as described by Downes et al. (1982). Data presented are means ± SE of three separate experiments.

In those experiments with exogenous substrates, platelet membranes (0.25–1 mg of protein) were incubated with small unilamellar vesicles (SUV) (25–50 μg of phospholipid) containing either [^{3}H]PI/PE (1:0.4 mol %) or [^{3}H]PIP_2/PE (1:0.4 mol %). The vesicles were prepared as described previously (Baldassare & Fisher, 1986b). The final assay buffer was 100 mM Tris–maleate, 80 mM KCl, and 2 mM $MgCl_2$ (Baldassare & Fisher, 1986b). Reactions were started by the addition of substrate and incubated for 2 min. The reaction was stopped as described above, and 500 μL of the upper aqueous phase was removed for liquid scintillation counting. The data are mean values ± SE of at least three separate experiments.

Table I: Release of Inositol Phosphates from [^{3}H]Inositol-Labeled Human Platelet Plasma Membranes[a]

additions	[^{3}H]inositol phosphates		
	IP	IP_2	IP_3
none	85 ± 73	140 ± 89	107 ± 76
GTPγS (1 μM)	78 ± 62	188 ± 73	119 ± 72
GTPγS (100 μM)	93 ± 64	358 ± 98	385 ± 81
thrombin (1 unit/mL)	98 ± 75	173 ± 46	105 ± 34
GTPγS (1 μM) + thrombin (1 unit/mL)	84 ± 44	411 ± 85	354 ± 87
Ca^{2+} (1 mM)	89 ± 38	741 ± 93	737 ± 99

[a] [^{3}H]Inositol-labeled platelet membranes (150 μg) containing approximately 31 × 10^4 cpm/mg of protein were incubated with the indicated agents, as described under Experimental Procedures. Unless stated otherwise, the concentration of free Ca^{2+} was adjusted to 100 nM with Ca^{2+}/EGTA as described under Experimental Procedures. The inositol phosphates were analyzed by Dowex chromatography.

Pertussis Toxin Treatment. Platelet membranes were ADP-ribosylated as described by Halenda et al. (1986). Briefly, platelet membranes were incubated for 30 min in 20 mM Hepes, 100 mM KCl, and 5 mM $MgCl_2$, pH 7.0, containing 1 mM each of dithiothreitol, thymidine, GTP, ATP, and NAD plus 50 μg/mL pertussis toxin. The extent of the reaction was determined by first separating the proteins by SDS–PAGE and then measuring the incorporation of [^{32}P]-NAD by radioautography. The reaction was 85–90% complete within 30 min (data not shown). The pertussis toxin treated membranes were then centrifuged at 100000*g* for 1 h at 4 °C and resuspended in buffer A. Control membranes were taken through the same incubation procedure but without pertussis toxin.

Protein Content. Protein was determined as described by Bradford et al. (1985) using bovine serum albumin as the standard.

Results

Inositol Phosphate Formation by [^{3}H]Inositol-Labeled Platelet Plasma Membranes. The platelet membranes used throughout the course of these studies contained less than 1% of the total lactate dehydrogenase activity found in platelet sonicate. In addition, the membrane-associated PLC activity could be solubilized only after the addition of detergent (1% DOC). These data indicate that the membrane-associated PLC activity is tightly associated with the membrane and does not represent cytosolic contamination. These data are in agreement with the results of Banno and Nozawa (1987), who recently reported a membrane-associated PLC activity that was solubilized only by the addition of detergent. Table I demonstrates the formation of inositol phosphates from ^{3}H-labeled platelet plasma membranes in response to GTPγS, thrombin, and calcium. Low concentrations of neither GTPγS (less than 1 μM) nor thrombin (1 unit/mL) stimulated the release of inositol phosphates. However, a higher concentration of GTPγS (100 μM) increased release of IP_2 and IP_3 approximately 3-fold but did not significantly increase accumulation of IP. A similar 3-fold rise in IP_2 and IP_3 was observed with thrombin (1 unit/mL) plus GTPγS (1 μM). This synergistic effect of thrombin plus GTPγS was specific for the formation of IP_2 and IP_3. Addition of calcium (1 mM) also resulted in significant accumulation of IP_2 and IP_3 without a detectable increase in IP. The inability to detect IP release was not due to the distribution of radiolabel among the phosphoinositides, since [^{3}H]inositol was found to be 86% PI, 5% PIP, 9% PIP_2. These data suggest that the GTPγS-dependent thrombin-stimulated phospholipase C activity in platelet plasma membranes is specific for PIP_2 and PIP. We

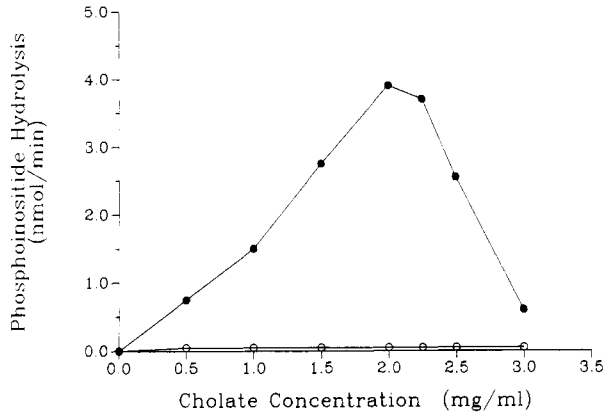

FIGURE 1: Dependence of PI and PIP_2 hydrolysis by membrane-associated phospholipase C on sodium cholate concentration. Human platelet membranes (1 mg of protein) were incubated with lipid vesicles containing either [^{3}H]PI (○) or [^{3}H]PIP_2 (●) and the indicated concentrations of sodium cholate. Free Ca^{2+} concentrations were maintained at 100 nM with Ca^{2+}/EGTA as described under Experimental Procedures. Phosphoinositide hydrolysis was quantitated as described under Experimental Procedures. Results are mean values of three separate experiments.

therefore undertook to investigate the substrate specificity of this reaction.

Hydrolysis of Exogenous PI and PIP_2 by Platelet Plasma Membrane PLC. One approach to address the substrate specificity of the thrombin-stimulated PLC activity is to utilize exogenous PI and PIP_2 as substrates. This allows a systematic evaluation of the relative rates of hydrolysis of the two different phosphoinositides. Previous reports (Jackowski et al., 1986; Rock & Jackowski, 1987) have shown that agonist-dependent hydrolysis of exogenous phosphoinositides may require addition of detergent. We, therefore, examined the effect of cholate on thrombin-stimulated hydrolysis of exogenous PI and PIP_2 by platelet plasma membranes. In the absence of detergent, no hydrolysis of either phosphoinositide was observed (Figure 1). Addition of cholate to 0.2% maximally stimulated PIP_2 hydrolysis (Figure 1). Higher concentrations of detergent resulted in decreased thrombin-stimulated enzyme activity. This loss of thrombin-stimulated PLC activity was not due to inhibition of the enzyme by detergent, since thrombin-independent PIP_2 hydrolysis, measured in the presence of 1 mM Ca^{2+}, increased linearly between 0 and 0.4% cholate (data not shown). Between 0 and 0.4% cholate, no thrombin-stimulated hydrolysis of PI was observed (Figure 1).

The effects of thrombin and GTPγS on the hydrolysis of exogenous PIP_2 and PI by the membrane-associated PLC, in the presence of submicromolar calcium, are shown in Table II. Neither thrombin (1 unit/mL) alone nor low concentrations of GTPγS (1 μM) stimulated the breakdown of PIP_2. GTPγS at 100 μM, however, resulted in significant PIP_2 degradation. GTPγS (1 μM) plus thrombin (1 unit/mL) synergistically activated PIP_2 hydrolysis. In contrast, with PI as exogenous substrate, significantly less PLC activity was observed. These data are consistent with those in Table I showing the release of IP_2 and IP_3, but not IP, from labeled membranes. Thus, the platelet plasma membrane associated PLC activity, when activated by thrombin and GTPγS, preferentially hydrolyzes PIP_2.

Table III demonstrates the nucleotide specificity of stimulation of membrane-bound PIP_2–PLC activity. In the presence of thrombin (1 unit/mL), GTPγS stimulated PIP_2 hydrolysis by the membrane associated PLC approximately 10-fold. Thrombin-induced PIP_2 hydrolysis by platelet plasma membranes was also synergistically activated by GTP, although

Table II: Hydrolysis of Exogenous PI and PIP_2 by Platelet Plasma Membranes[a]

additions	hydrolysis [^{3}H]PIP_2 (cpm)	hydrolysis [^{3}H]PI (cpm)
none	300 ± 50	291 ± 67
GTPγS (1 μM)	438 ± 125	328 ± 117
GTPγS (100 μM)	2914 ± 168	285 ± 126
thrombin (1 unit/mL)	289 ± 95	309 ± 130
GTPγS (1 μM) + thrombin (1 unit/mL)	3387 ± 114	299 ± 184

[a] Platelet membranes (250 μg) were incubated at 37 °C with [^{3}H]-PIP_2/PE or [^{3}H]PI/PE (1:0.4 mole ratio) phospholipid vesicles (50 μg of phospholipid, 25 × 10^3 cpm) in the presence of 0.25% cholate plus 100 nM Ca^{2+}. Free Ca^{2+} concentrations were maintained with Ca^{2+}/EGTA as described under Experimental Procedures. After 5 min, the reaction was terminated, and water-soluble [^{3}H]inositol phosphates were determined as described under Experimental Procedures.

Table III: Nucleotide Specificity of Hydrolysis of Exogenous PIP_2 by Platelet Plasma Membranes[a]

additions	PIP_2 hydrolysis [nmol min^{-1} (mg of protein)$^{-1}$]
none	0.57 ± 0.15
GTPγS (1 μM)	5.27 ± 0.31
GTP (100 μM)	2.56 ± 0.32
GDPβS (100 μM)	0.50 ± 0.18
ATP (100 μM)	0.42 ± 0.16
UTP (100 μM)	0.51 ± 0.14

[a] Platelet membranes (50 μg) were incubated at 37 °C with [^{3}H]-PIP_2/PE (1:0.4 mole ratio) phospholipid vesicles (50 μg of phospholipid, 30 × 10^3 cpm) in the presence of 0.25% cholate and 100 nM Ca^{2+} plus thrombin (1 unit/mL). Guanine and adenine nucleotides were added at the indicated concentrations. Water-soluble [^{3}H]inositol phosphates were determined as described under Experimental Procedures.

to a lesser extent than GTPγS. ATP, UTP, and the nonhydrolyzable GDP analogue GDPβS did not increase PIP_2 hydrolysis in the presence of thrombin. Furthermore, the nonhydrolyzable GDP analogue GDPβS markedly inhibited the ability of GTP or GTPγS to stimulate PIP_2 hydrolysis (data not shown).

Calcium and pH Dependencies of Membrane-Associated PLC. Previous reports (Baldassare et al., 1988; Hofmann & Marjerus, 1982; Low et al., 1986; Ryu et al., 1986; Siess & Lapetina, 1983) have demonstrated that the soluble PI-specific PLC from human platelets requires millimolar calcium for maximal PI hydrolysis. We, therefore, examined the calcium dependence of PI and PIP_2 hydrolysis by the membrane-associated enzyme. PLC-catalyzed hydrolysis of PIP_2 was stimulated by calcium in a dose-dependent fashion. Maximal activity occurred at 10 μM calcium. In contrast, little hydrolysis of PI was observed at 10 μM calcium. Maximal PI hydrolysis occurred between 500 μM and 1 mM calcium (Figure 2).

The pH dependence for both calcium- and GTPγS-dependent PIP_2 hydrolysis was quite similar (Figure 3). Optimal activity occurred between 6.5 and 7.0.

Effect of Pretreatment with Pertussis Toxin and TPA on Membrane-Associated PLC Activity. Several studies have demonstrated that pretreatment of permeabilized platelets (Brass et al., 1986) and platelet membranes (Houslay et al., 1986; O'Rourke et al., 1987) with pertussis toxin, and of intact platelets with TPA (Zavoico et al., 1985; Rittenhouse & Sasson, 1985; Kikuchi et al., 1986), inhibits thrombin-stimulated hydrolysis of endogenous phosphoinositides. Thrombin-induced hydrolysis of exogenous PIP_2 by isolated platelet plasma membranes was similarly inhibited by pertussis toxin and TPA (Table IV). Pertussis toxin, however, did not inhibit

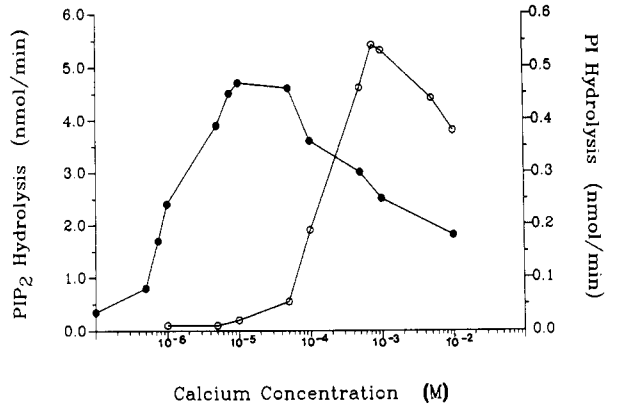

FIGURE 2: Calcium concentration dependence of platelet membrane associated phospholipase C activity. Phospholipid vesicles containing either [^{3}H]PI or [^{3}H]PIP_2 platelet membranes (1 mg of protein) were incubated with Tris–maleate buffer (pH 7.0) plus 0.25% cholate. Calcium concentrations were maintained with Ca^{2+}/EGTA buffers as described under Experimental Procedures. Inositol phosphates were quantitated as described under Experimental Procedures. The data are expressed as mean values for three separate experiments. (○) PI; (●) PIP_2.

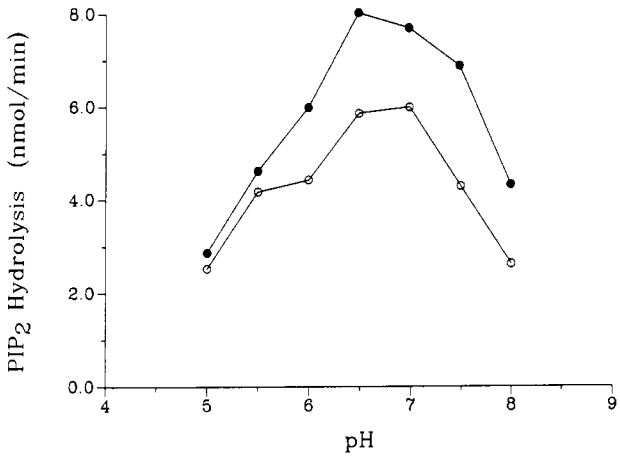

FIGURE 3: pH dependence of calcium- and thrombin-stimulated PIP_2 hydrolysis by membrane-associated PLC. Membranes (1 mg) were incubated with [^{3}H]PIP_2-containing lipid vesicles in 100 mM Tris–maleate buffer at the indicated pH values. Unless stated otherwise, Ca^{2+} concentrations were adjusted to 100 mM with Ca^{2+}/EGTA buffers as described under Experimental Procedures. The inositol phosphates were determined as described under Experimental Procedures. (○) Thrombin (1 unit/mL) plus GTPγS (1 μM); (●) 10 μM calcium.

activation of membrane-associated PLC by GTPγS (100 μM) or calcium (1 mM). TPA treatment inhibited GTPγS-dependent PLC activity but had no effect on calcium-stimulated activity.

Similar effects of pertussis toxin and TPA on phosphoinositide hydrolysis in [^{3}H]inositol-labeled membranes from a variety of cell types have been reported (Rittenhouse & Sasson, 1985; Zavoico et al., 1985; Kikuchi et al., 1987; Majerus et al., 1986; Orellana et al., 1987; Smith et al., 1987). These results suggest that pertussis toxin disrupts the coupling between the agonist receptor and G-protein activation, while TPA inhibits the coupling to PLC to the activated G-protein.

Discussion

The above data demonstrate that platelet plasma membranes contain an integral membrane PLC activity that is synergistically stimulated by thrombin and GTPγS under physiological concentrations of calcium and magnesium. The enzyme catalyzed the formation of IP_2 and IP_3 but not IP from endogenous ^{3}H-labeled phosphoinositides. Even in the presence of 1 mM calcium IP_2 and IP_3, but not IP, formation was observed. This is surprising, since soluble phospholipase C, which is the predominant PLC in human platelets, readily hydrolyzes all three phosphoinositides in the presence of millimolar calcium. This suggests that the membrane-associated PLC and the soluble PLC have different catalytic properties. We therefore utilized exogenous PI- and PIP_2-containing lipid vesicles to investigate the substrate specificity of the membrane-associated PLC. The results of these experiments clearly demonstrated that at submicromolar calcium, thrombin plus GTPγS stimulated PIP_2 but not PI hydrolysis by the membrane-associated PLC. A similar substrate specificity was observed in the presence of millimolar calcium. We conclude that in the presence of high concentrations of calcium the membrane-associated and soluble PLC activities in human platelets have distinct substrate specificities.

Table IV: Inhibition of Thrombin-Stimulated PIP_2 Hydrolysis by Pertussis Toxin and TPA[a]

additions	PIP_2 hydrolysis [nmol min^{-1} (mg of protein)$^{-1}$]		
	no pretreatment	pertussis toxin	pretreatment with TPA
none	0.26 ± 0.12	0.25 ± 0.10	0.20 ± 0.11
GTPγS (1 μM) + thrombin (1 unit/mL)	5.08 ± 0.56	0.64 ± 0.26	0.52 ± 0.25
GTPγS (1 mM)	5.80 ± 0.41	5.68 ± 0.36	0.67 ± 0.30
Ca^{2+} (1 mM)	7.78 ± 0.32	7.48 ± 0.53	5.38 ± 0.37

[a] Isolated platelet plasma membranes were incubated with pertussis toxin (100 ng/mL) plus NAD. Intact platelets were treated with 100 nM TPA and membranes prepared from the pretreated platelets as described under Experimental Procedures. The pertussis toxin and TPA-pretreated membranes (240 μg of protein) were incubated at 37 °C with PIP_2/PE (1:0.4 mole ratio) phospholipid vesicles (50 μg of phospholipid, 30 000 cpm) in the presence of 0.25% cholate. Unless stated otherwise, Ca^{2+} concentrations were maintained at 100 nM with Ca^{2+}/EGTA buffers as described under Experimental Procedures. Inositol phosphates were determined as described under Experimental Procedures.

Membrane-associted PLC activities in HL-60 cells (Kikuchi et al., 1987), rabbit platelets (Hrbolich et al., 1987), and human (Rebecchi & Rosen, 1987) and hamster (Magnaldo et al., 1987) fibroblasts have also been reported to preferentially degrade polyphosphoinositides in response to agonists and high concentrations of calcium. Whether this is a general property of agonist-coupled membrane-associated PLC is not presently known.

In most tissues (Hofmann & Marjerus, 1982; Irvine et al., 1979; Ryu et al., 1986), including platelets (Baldassare et al., 1988; Banno & Nozawa, 1987; Siess & Lapetina, 1983), the majority of phospholipase C activity is soluble. It has been argued that the phospholipase C activity associated with the plasma membrane is due, in fact, to contamination by the soluble enzyme (Siess & Lapetina, 1983). Banno et al. (1987) have demonstrated, however, the presence of membrane-associated PLC activity that is not due to cytoplasmic contamination. The difference in reactivity toward PI between the soluble and membrane-associated enzymes raises the possibility that the two enzymes are distinct. To address this issue requires purification and characterization of the membrane-associated PLC.

There exists extensive evidence that agonist activation of PLC leads to hydrolysis of PIP_2. We have demonstrated that platelet membranes contain thrombin-stimulated PLC activity that specifically hydrolyzes PIP_2. It is likely, therefore, that this activity participates in polyphosphoinositide-mediated

signal transduction. A number of studies with intact cells (Majerus et al., 1986), including platelets (Wilson et al., 1985), have suggested, however, that direct hydrolysis of PI occurs in response to agonist activation. Our data lead us to conclude that if agonist-stimulated PI hydrolysis occurs in platelets, it is not catalyzed by the membrane-associated PLC activity.

Registry No. GTP, 86-01-1; GTPγS, 37589-80-3; thrombin, 9002-04-4; phosphatidylinositol phospholipase C, 63551-76-8.

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