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Effect of Acidic Phospholipids on Sphingosine Kinase

Ana Olivera, Jutta Rosenthal, and Sarah Spiegel

Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC 20007

Abstract Sphingosine-1-phosphate (SPP) is a unique sphingolipid metabolite involved in cell growth regulation and signal transduction. SPP is formed from sphingosine in cells by the action of sphingosine kinase, an enzyme whose activity can be stimulated by growth factors. Little is known of the mechanisms by which sphingosine kinase is regulated. We found that acidic phospholipids, particularly phosphatidylserine, induced a dose-dependent increase in sphingosine kinase activity due to an increase in the apparent Vmax of the enzyme. Other acidic phospholipids, such as phosphatidylinositol, phosphatidic acid, phosphatidylinositol bisphosphate, and cardiolipin stimulated sphingosine kinase activity to a lesser extent than phosphatidylserine, whereas neutral phospholipids had no effect. Diacylglycerol, a structurally similar molecule which differs from phosphatidic acid in the absence of the phosphate group, failed to induce any changes in sphingosine kinase activity. Our results suggest that the presence of negative charges on the lipid molecules is important for the potentiation of sphingosine kinase activity, but the effect does not directly correlate with the number of negative charges. These results also support the notion that the polar group confers specificity in the stimulation of sphingosine kinase by acidic glycerophospholipids. The presence of a fatty acid chain in position 2 of the glycerol backbone was not critical since lysophosphatidylserine also stimulated sphingosine kinase, although it was somewhat less potent. Dioleoylphosphatidylserine was the most potent species, including a fourfold stimulation, whereas distearoyl phosphatidylserine was completely inactive. Thus, the degree of saturation of the fatty acid chain of the phospholipids may also play a role in the activation of sphingosine kinase.

Key words: phospholipids, phosphatidylserine, sphingosine, sphingosine-1-phosphate, sphingosine kinase

Sphingosine kinase, the enzyme that catalyzes the phosphorylation of sphingoid bases[1] on their primary hydroxyl groups, was suggested to be the rate-limiting enzyme involved in the catabolism of sphingolipids [Keenan and Haegelin, 1969; Stoffel et al., 1970]. Sphingosine kinase has been found in yeast [Stoffel et al., 1968], *tetrahymena pyriformis* [Keenan, 1972], rat liver, kidney, and brain [Buehrer and Bell, 1992; Keenan and Haegelin, 1969], bovine brain [Louie et al., 1976], and human and porcine platelets [Buehrer and Bell, 1992; Stoffel et al., 1973]. Its ubiquitous presence suggests an important physiological role for this enzyme, since, otherwise, evolutionary pressures would have likely resulted in its disappearance. Our studies indicate that sphingosine kinase may have a function in signal transduction, producing the novel lipid messenger, sphingosine-1-phosphate (SPP) [Olivera and Spiegel, 1993]. When added exogenously, SPP initiates complex intracellular actions that lead to DNA synthesis and cell division [Zhang et al., 1991], including inositol 1,4,5-trisphosphate–independent release of calcium from internal sources [Ghosh et al., 1990, 1994; Mattie et al., 1994], phospholipase D activation [Desai et al., 1992], and stimulation of the Raf/MKK/MAP kinase signaling pathway [Wu et al., 1995]. More importantly, we found that PDGF or serum stimulated cytosolic sphingosine kinase activity and induced formation of SPP in Swiss 3T3 fibroblasts [Olivera and Spie-

[1]*Sphingoid base* is used as a general term for sphingosine, sphinganine, and phytosphingosine and their homologs. The generic term *sphingosine* refers only to the 18 carbon, long chain base with a 4-*trans* double bond.

Abbreviations used: BSA, bovine serum albumin; CL, cardiolipin; DAG, diacylglycerol; DMEM, Dulbecco's modified Eagle's medium; PA, phosphatidic acid; PBS, phosphate buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIP_2, phosphatidylinositolbisphosphate; PKC, protein kinase C; PLD, phospholipase D; PMSF, phenylmethyl sulfonyl fluoride; PS, phosphatidylserine; SM, sphingomyelin; SPP, sphingosine-1-phosphate; TLC, thin layer chromatography.

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Address reprint requests to Sarah Spiegel, Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, 357 Basic Science Building, 3900 Reservoir Road NW, Washington, DC 20007.

gel, 1993]. Inhibition of the PDGF-induced formation of SPP attenuated cellular proliferation induced by PDGF, indicating that SPP might play an important role in cellular proliferation induced by this growth factor [Olivera and Spiegel, 1993]. Sphingosine kinase activity has also been shown to be stimulated by other growth promoting agents such as the tumor promoter, 12-O-tetradecanoylphorbol 13-acetate (TPA) [Mazurek et al., 1994]. Thus, the regulation of sphingosine kinase might be a crucial but yet unexplored step in mitogenic signaling.

Although sphingosine kinase activity has been found in the cytosol of a wide variety of cells and tissues [Hirschberg et al., 1970; Louie et al., 1976; Olivera and Spiegel, 1993; Olivera et al., 1994b; Stoffel et al., 1973], there is some indication of membrane-bound forms [Buehrer and Bell, 1992; Ghosh et al., 1994; Keenan, 1972]. The mechanism of activation of sphingosine kinase by external stimuli or the cellular compartments where sphingosine kinase interacts with its substrate, sphingosine, is not known. Free sphingosine is mainly associated with the plasma membrane, although it can move rapidly across and between membranes [Merrill, 1991]. Thus, it is likely that upon activation with PDGF or other growth factors, cytosolic sphingosine kinase interacts with sphingosine in the vicinity of the plasma membrane [Buehrer and Bell, 1992] or in the endoplasmic reticulum [Ghosh et al., 1994]. Numerous enzymes involved in signal transduction are recruited to membranes through protein-protein or protein-lipid interactions, and some of them can be modulated by membrane lipid cofactors. Here, we have examined the effects of the major phospholipid components of cell membranes and of lipid second messengers formed upon cell activation on the activity of sphingosine kinase.

MATERIALS AND METHODS

Materials

[γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Sphingosine was from Matreya (Pleasant Gap, PA). Lipids were from Avanti Polar Lipids (Birmingham, AL) or Serdary Research Laboratories (Englewood Cliffs, NJ). Triton X-100 was purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Silica gel 60 G plates were from EM Sciences (Cherry Hill, NJ). Standard SPP was prepared by enzymatic digestion of sphingosylphosphorylcholine with phospholipase D as previously described [Zhang et al., 1991].

Preparation of Sphingosine Kinase

Swiss 3T3 fibroblasts from American Type Culture Collection, Rockville, MD, (CCL 92) were cultured as described [Spiegel, 1989]. Confluent Swiss 3T3 fibroblasts were washed with cold PBS and scraped in 20 mM Tris buffer (pH 7.4) containing 20% glycerol, 1 mM mercaptoethanol, 1 mM EDTA, 1 mM sodium orthovanadate, 15 mM NaF, 10 μg/ml leupeptin and aprotinin, 1 mM PMSF, and 0.5 mM 4-deoxypyridoxine. Cells were disrupted by freeze-thawing and the cytosolic fraction prepared by ultracentrifugation at 105,000*g* for 90 min as previously described [Olivera and Spiegel, 1993; Olivera et al., 1994b]. The preparations were stored at −70°C, and sphingosine kinase activity was stable for several months.

Lipids. Known amounts of various lipids were dried under a stream of N_2. For the preparation of vesicles, lipids were suspended in distilled water and probe-sonicated using four 20 s bursts. For the preparation of mixed micelles, lipids were solubilized in 3–6% Triton X-100 by vortexing and brief probe sonication. The lipid vesicles or micelle preparations were incubated for 5–10 min at room temperature before use.

Sphingosine kinase activity. For studies of ion requirements, varying concentrations of different divalent cations ranging from 0.25–1 mM were added to 50 μg of Swiss 3T3 cytosol, containing sphingosine kinase activity, without or with sphingosine (50 μM), delivered as sphingosine-BSA complex [Olivera et al., 1994a]. The reaction mixtures contained 20 mM Tris buffer (pH 7.4), 5% glycerol, 1 mM mercaptoethanol, 0.25 mM EDTA, 1 mM sodium orthovanadate, 15 mM NaF, 10 μg/ml leupeptin and aprotinin, 1 mM PMSF, and 0.5 mM 4-deoxypyridoxine in a final volume of 200 μl. Reactions were started by addition of 10 μl of [γ^{32}P]ATP (1–2 μCi, 20 mM) and incubated for 30 min at 37°C. Reactions were terminated by addition of 20 μl of 1 N HCl followed by 0.8 ml of chloroform/methanol/HCl (100:200:1). After vigorous vortexing, 240 μl of chloroform and 240 μl of 2 N KCl were added and phases separated by centrifugation. The labelled lipids in the organic phase were resolved by TLC on silica gel G60 with 1-butanol/methanol/acetic acid/water (80:20:10:20) and visualized by autoradiography. The radioactive spots corresponding to authentic SPP were iden-

tified as described [Zhang et al., 1991], scraped from the plates, and counted in a scintillation counter. Sphingosine kinase activity was expressed as picomoles of SPP formed per milligram of protein per minute. To examine lipid requirements, two different types of substrate preparations were used. In the first, various amounts of different lipids (from 1.5–25 nmol) and sphingosine (50 μM) were added as sonicated lipid vesicles. In the micellar preparation, different lipids were added as Triton X-100 mixed micelles, containing up to 12 moles of lipid per mole of Triton X-100, and 2.5 mol% sphingosine. The final concentration of Triton X-100 in the reaction mixtures was 0.3%. Sphingosine kinase assays were carried out as described above, in the presence of [γ^{32}P]ATP (1 mM) and $MgCl_2$ (0.5 mM), unless indicated otherwise.

RESULTS AND DISCUSSION

Effects of Divalent Cations on Sphingosine Kinase Activity

Divalent cations have previously been shown to be required for the activation of other kinases known to play an important role in signal transduction (i.e., diacylglycerol kinase, phosphatidylinositol 3 kinase, protein kinase C) together with lipid cofactors and to modify the effectiveness of the lipid cofactor [Carpenter et al., 1990; Orr and Newton, 1992b; Walsh and Bell, 1986]. We examined the dependency of sphingosine kinase activity on various divalent ions to define proper conditions for the studies of its activity in the absence or presence of various lipids. As shown in Figure 1, sphingosine kinase activity was found to be maximal at a Mg^{2+} concentration of 0.75 mM. Concentrations greater than 3 mM were inhibitory (data not shown). Similar results were observed by Louie et al. [1976] for partially purified bovine brain sphingosine kinase. Optimum concentrations of $MnCl_2$ were 40% less effective in stimulating sphingosine kinase than the optimal $MgCl_2$ concentrations (Fig. 1A). Addition of various concentrations of $MnCl_2$ in the presence of an optimal concentration of $MgCl_2$ did not further enhance activity (Fig. 1A). Calcium had almost no effect on activity, whereas other metallic ions, especially zinc and copper, inhibited sphingosine kinase activity in the absence (Fig. 1B) or the presence of $MgCl_2$ (data not shown). Thus, it seems that magnesium is the only metal ion required for sphingosine kinase activity, as has been shown for a number of similar kinases that phosphorylate lipids.

Effects of Cellular Phospholipids on Sphingosine Kinase Activity

We have recently found that crude cellular lipids extracted from diverse cell types enhance

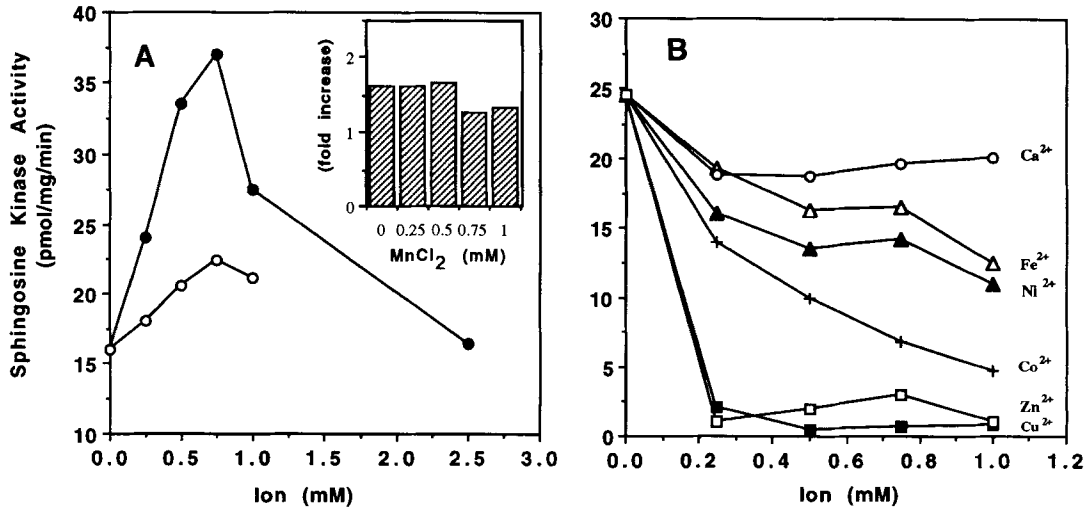

Fig. 1. Effect of various divalent metal cations on sphingosine kinase activity. Sphingosine kinase activity was measured as described in Materials and Methods in the presence of 0.25 mM EDTA and in the absence of $MgCl_2$, unless indicated. **A:** Concentration-dependent activation of sphingosine kinase by $MgCl_2$ (*closed circles*) and $MnCl_2$ (*open circles*). **Inset:** Effect of increasing concentrations of $MnCl_2$ on sphingosine kinase in the presence of an optimal concentration of $MgCl_2$ (0.5 mM). Results are expressed as fold stimulation with respect to sphingosine kinase activity determined in the absence of metal ions. **B:** Effect of other divalent metal cations on sphingosine kinase activity in the absence of $MgCl_2$. Results are from a representative experiment, and similar results were obtained in three independent experiments.

the ability of sphingosine kinase to phosphorylate sphingosine [Olivera et al., 1994a]. Consistent with our previous results, twofold stimulation of sphingosine kinase activity was observed after the addition of cellular lipids (containing 50 nmol of phospholipids) extracted from Swiss 3T3 fibroblasts (Fig. 2A). Since a growing number of kinases that play an important role in signal transduction are known to be regulated by phospholipids [Carpenter et al., 1990; Hannun et al., 1985; Jackowski and Rock, 1989; Orr and Newton, 1992b; Tsai et al., 1989], it was of interest to examine the effects of membrane phospholipids on sphingosine kinase activity. Among the phospholipids examined, only phosphatidylserine (PS) and phosphatidylinositol (PI) induced a marked increase in the phosphorylation of sphingosine in a dose-dependent manner (Fig. 2B). A detectable increase occurred at doses as low as 2 nmol of phospholipid, and a maximum effect was reached at 10–15 nmol of phospholipids. PS stimulated the activity by threefold compared to 2–2.5-fold with PI. In contrast, phosphatidylethanolamine (PE), phosphatidylcholine (PC), or sphingomyelin (SM) at concentrations up to 25 nmol had either no effect (PE) or slightly decreased (PC and SM) sphingosine kinase activity (Fig. 2B). Amounts of PE greater than 25 nmol induced a maximum of about twofold increase in sphingosine kinase activity (data not shown). Similarly, PKC, which requires PS for activation, has been reported to bind PE with lower affinity [Orr and Newton, 1992a]. Orr and Newton suggested that PE can interact with putative PS binding sites, probably due to the presence of the amine group [Orr and Newton, 1992a]. Interestingly, like sphingosine kinase, PKC can also bind sphingosine, probably by recognition of its headgroup which shares some similarity with the PS headgroup [Merrill, 1991]. Thus, our results may also suggest an involvement of the amine group of these phospholipids for binding to and/or activation of sphingosine kinase.

Phospholipids dispersed by sonication can exist in different aggregation states depending on the phospholipid species [Lichtenberg et al., 1983]. To rule out the possibility that differences in the phospholipid-mediated activation of sphingosine kinase activity were due to differences in the aggregation state of the individual phospholipid dispersions, PS (20 nmol) was sonicated together with either cellular lipid mixtures (containing 25 nmol of phospholipids) or

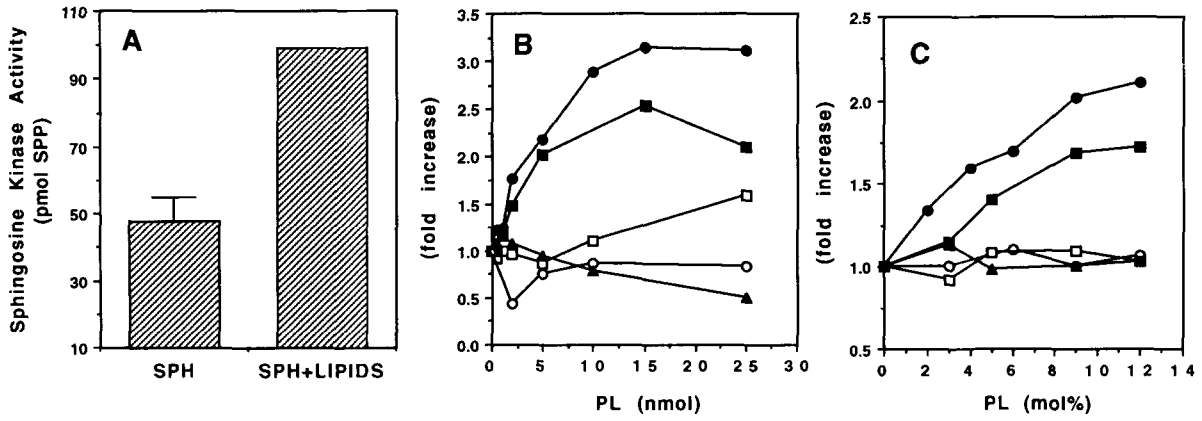

Fig. 2. Effect of cellular lipids and various phospholipids on sphingosine kinase activity. **A:** Potentiation of sphingosine kinase activity by cellular lipids isolated from Swiss 3T3 fibroblasts. Lipids were extracted from quiescent cultures, and phospholipid content was determined [Olivera et al., 1994a]. Cellular lipids containing 50 nmol of phospholipids were solubilized by sonication [Olivera et al., 1994a] and sphingosine kinase activity measured as described in Materials and Methods. **B:** Effect of different phospholipids on sphingosine kinase activity. Sphingosine kinase activity was measured in the presence of phospholipid vesicles and 50 μM sphingosine. The data are expressed as fold increase in sphingosine kinase activity determined in the absence of phospholipids (77 ± 3.5 pmol/min/mg). Results are from a representative experiment, and similar results were obtained in five independent experiments. **C:** Effect of Triton X-100/phospholipid mixed micelles on sphingosine kinase activity. Sphingosine (2.5 mol%) and increasing concentrations of phospholipids were solubilized in Triton X-100. Concentrations of phospholipids are expressed as mol% of Triton X-100. Results are from a representative experiment repeated four times, and data are expressed as fold increase of sphingosine kinase activity determined in the absence of phospholipids (129 ± 3.5 pmol/min/mg). PS, *closed circles;* PC, *open circles;* PI, *closed squares;* PE, *open squares;* SM, *triangles.*

PC (25 nmol), the most abundant neutral phospholipid found in cellular membranes. In both types of lipid mixtures, PS induced maximal potentiation of sphingosine kinase activity identical to that induced by sonicated PS alone (data not shown). To further substantiate the specific effect of these phospholipids, we measured sphingosine kinase activity in the presence of Triton X-100 micelles containing increasing amounts of phospholipids. Detergent-solubilized phospholipids exist as micelles, providing a more homogeneous and defined lipid system than vesicles obtained by sonication. Furthermore, mixed Triton X-100 and phospholipid micelles represent a widely used, reliable system for the study of protein activation by various lipids [Bazzi and Nelsestuen, 1987; Hannun et al., 1985; Lichtenberg et al., 1983; Newton and Koshland, 1990]. Consistent with the results shown in Figure 2B, only PS or PI stimulated sphingosine kinase activity when added as Triton X-100 mixed micelles (Fig. 2C). However, both PS and PI stimulated the kinase activity to a greater extent when they were presented to the enzyme as dispersed vesicles rather than as micelles (Fig. 2B compared to Fig. 2C). A similar preference for lipid in the form of liposomes vs. mixed micelles was described for the inhibitory effect of phosphatidic acid on ras-GTPase activating protein (GAP) [Tsai et al., 1989]. Even in the micellar assay, PS was more effective than PI, showing a potentiation at concentrations as low as 2 mol% and a maximal effect at 8–10 mol%. These concentrations are within the concentration range of these lipids found in biological membranes. The concentration of PS in the inner leaflet of cell membranes is 10–15 mol% [Nozawa et al., 1991]. The amount of PI in the plasma membrane is only 3 mol%, but intracellular membranes, such as endoplasmic reticulum, contain as much as 7 mol% [Nozawa et al., 1991]. Although conclusions about the physiological relevance of such activation or potential roles for different phospholipids in the activation of sphingosine kinase are premature, it is interesting to note that two forms of sphingosine kinase have been described: cytosolic and microsome-associated; a cytosolic form of the kinase could have access to plasma membrane PS, whereas the form associated with endoplasmic reticulum membranes [Ghosh et al., 1994] might interact with PI.

Effects of Acidic Phospholipids on Sphingosine Kinase Activity

Sphingosine kinase shows typical Michaelis Menten kinetics in the absence or presence of PS (Fig. 3). Addition of PS to sphingosine kinase preparations induced a threefold increase in the apparent Vmax (Fig. 3). Unlike PS, the stimulatory effects of PI depend on the sphingosine concentration: only at substrate concentrations higher than 30 μM was PI (20 nmol) able to stimulate sphingosine kinase activity, whereas at sphingosine concentrations lower than 30 μM PI was actually inhibitory (data not shown). These results suggest that the mechanism of sphingosine kinase activation by PS may be different from that of PI.

PS and PI are negatively charged molecules at neutral pH. Since there are many examples of enzymes that can bind to and be activated by acidic membrane phospholipids [Castuma et al., 1993; Tsai et al., 1989; Tsai et al., 1991; Walsh and Bell, 1986], it was of interest to examine the effects of other negatively charged phospholipids, added as either lipid vesicles or lipid micelles, on sphingosine kinase activity. As shown

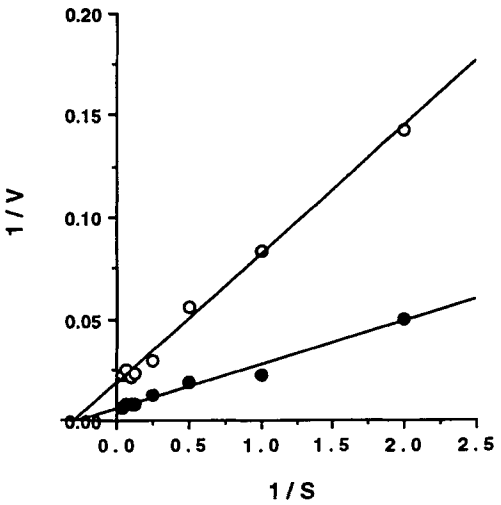

Fig. 3. Lineweaver-Burk plot of sphingosine kinase activity determined in the absence or presence of phosphatidylserine. Sphingosine kinase activity was determined in the presence of increasing concentrations of sphingosine in the absence (*open circles*) or in the presence of 20 nmol PS (*closed circles*). Velocity is expressed as picomoles of phosphorylated sphingosine formed per minute per milligram of protein and substrate concentrations as μM. Km values were 3.5 μM in the absence of phospholipids and 3.7 μM in the presence of PS; Vmax values were 55.5 pmol/min/mg protein and 175 pmol/min/mg protein, respectively.

in Figure 4A, in addition to PS and PI, phosphatidic acid (PA), phosphatidylinositolbisphosphate (PIP_2), and cardiolipin (CL), in either vesicle or micellar forms, were also effective in stimulating sphingosine kinase activity. The stimulatory effect of PA occurred over a low and narrow concentration range (less than 5 μM) (Fig. 4B), whereas the concentration-dependent activation by CL (Fig. 4B) or PIP_2 (not shown) was saturable and resembled the effects of PS or PI (Fig. 2B). Although PIP_2 and CL are the most negatively charged molecules among all the lipids tested, they were not as effective as PS. Thus, the presence of negative charges on the lipid molecules seems to be important for the potentiation of sphingosine kinase activity, but there is no direct correlation between the number of negative charges and their effects on sphingosine kinase activity.

Consistent with the importance of negatively charged groups, diacylglycerol (DAG) (dioleoyl, dioctanoyl, or dipalmitoyl), which differs from PA in the absence of the phosphate group in third position of the glycerol backbone, failed to induce any change in sphingosine kinase activity. DAG has been shown to potently synergize with PS in the activation of PKC, especially in the presence of calcium [Hannun et al., 1985; Nishizuka, 1992]. However, DAG, even in the presence of PS and calcium, did not have any stimulatory effects on sphingosine kinase activity. Within the framework of signal transduction, it is interesting to note that the PLC-derived second messengers, DAG and calcium, may play a role in activating PKC α, β, and γ isoforms [Hannun et al., 1985] but probably not in the sphingosine kinase-signaling pathway, whereas the PLD-derived second messenger PA [Exton, 1990], at very low concentrations (lower than 2 μg/ml), is able to modify sphingosine kinase activity. This concentration of PA is even lower than that required to inhibit Ras-GAP activity [Tsai et al., 1989]. Although it is difficult to estimate the concentration of PA in membrane microenviroments, cellular PA levels can increase in response to diverse mitogens. The product of sphingosine kinase, SPP, is a strong activator of PLD [Desai et al., 1992] and increases PA levels in cells [Desai et al., 1992; Zhang et al., 1991]. As shown in Figure 4B, the effective concentration range of PA for stimulation of sphingosine kinase is very narrow and might provide a potential fine feedback control mechanism between PLD and sphingosine kinase activity.

Effect of Fatty Acid Composition on Phospholipid Stimulation of Sphingosine Kinase Activity

We also investigated the importance of the fatty acid chains esterified to the hydroxyl groups at positions 1 and 2 on PS-induced sphingosine kinase stimulation with the aid of chemically synthesized PS derivatives. Dioleoyl-PS was the most potent species, inducing a fourfold stimulation of sphingosine kinase activity (Fig. 5A). Similar to the effect of natural PS from bovine

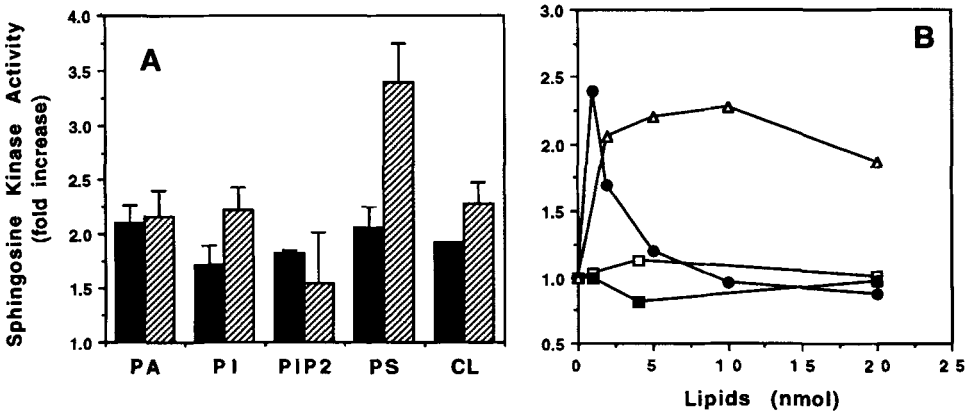

Fig. 4. Stimulation of sphingosine kinase activity by acidic phospholipids. **A:** Negatively charged lipids were added in Triton X-100 mixed micelles (*solid bars*) or as sonicated vesicles (*hatched bars*) and sphingosine kinase activity measured as described in Materials and Methods. The concentrations in the standard vesicular assay were as follows: PA, 2 nmol; PI, PIP2, and PS, 25 nmol; and CL, 10 nmol. In the micellar assay they were as follows: PA and CL, 2.5 mol%; and PI, PIP_2, and PS, 10 mol%. Results are the mean ± SD of two different experiments. **B:** Sphingosine kinase activity was measured in the presence of increasing amounts of PA (*closed circles*), dioleoylDAG (*open squares*), dioctanoylDAG (*closed squares*), and CL (*open triangles*).

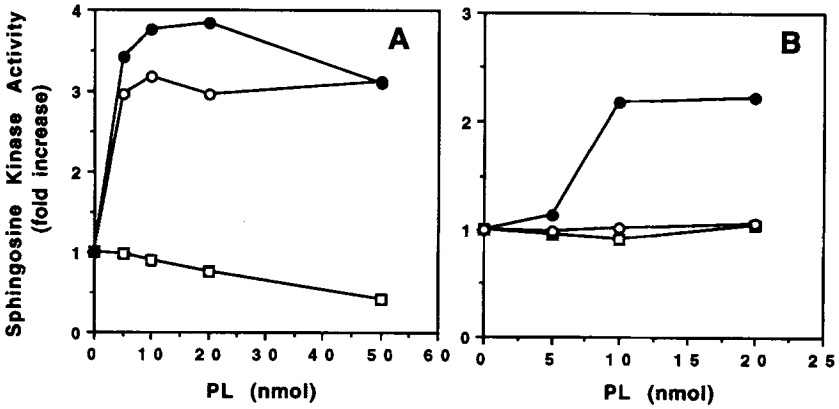

Fig. 5. Fatty acid content and composition affects phosphatidyl serine stimulation of sphingosine kinase. **A:** Sphingosine kinase activity was determined in the presence of increasing amounts of chemically synthesized PS containing different fatty acid chains delivered as sonicated lipid vesicles: 1,2-dioleoylPS (*closed circles*), 1-stearoyl, 2-oleoylPS (*open circles*), and 1,2-distearoylPS (*open squares*). Results are from a representative experiment repeated four times, and data are expressed as fold increase in the sphingosine kinase activity determined in the absence of phospholipids. **B:** Sphingosine kinase activity was measured in the presence of increasing amounts of 1-acyl, 2-sn-glycero-phospho-L-serine (lysoPS) (*closed circles*), 1-acyl, 2-sn-glycero-phospho-L-ethanolamine (lysoPE) (*open squares*), and 1-acyl, 2-sn-glycero-phospho-L-choline (lysoPC) (*open circles*) added as sonicated lipid vesicles.

brain which contains 41% stearoyl and 31% oleoyl as fatty acid chains, 1-stearoyl, 2-oleoyl-PS induced a two- to threefold stimulation, whereas distearoyl-PS was completely inactive (Fig. 5A). A specificity in the degree of saturation of phospholipid fatty acid chains has been observed for the inhibitory effect of PA on GAP activity [Tsai et al., 1989]. Ras-GAP activity was inhibited by PA with saturated fatty acids, whereas unsaturated ones were virtually inactive in liposomes or mixed micelle systems. The absence of a fatty acid chain in position 2 of the glycerol backbone and the presence of mainly stearoyl in position 1 (lysoPS) did not abrogate the potentiating activity of PS, although it was somewhat less potent (twofold increase compared to threefold for the parent PS) (Fig. 5B). These results contrast with the structural lipid requirements for PKC, since lysoPS, unlike PS, did not stimulate PKC, indicating a greater dependency of PKC on the interfacial phospholipid conformation [Lee and Bell, 1989] than that of sphingosine kinase. It is important to mention that the effect of lysoPS was specific and not due to detergent-like effects since lysoPE or lysoPC, added as either vesicles (Fig. 5B) or as micelles, had no effects on sphingosine kinase activity. Only lysoPS (Fig. 5B) or lysoPI (not shown), similar to their glycerophospholipid counterparts, were able to stimulate sphingosine kinase activity by twofold.

CONCLUDING REMARKS

Our results suggest that the polar group confers specificity to the stimulation of sphingosine kinase by acidic glycerophospholipids, and the presence of unsaturated fatty acid chains favors these effects, while saturated fatty acid chains in both positions of the glycerol backbone abolish the stimulatory effects. The mechanism by which acidic phospholipids enhance the phosphorylating activity of sphingosine kinase needs to be further investigated. Some interesting possibilities involve either direct or indirect enzyme-acidic phospholipid interactions or phospholipid-substrate interactions. Sphingosine, at physiological pH, exists mainly as protonated and neutral forms [Lopez-Garcia et al., 1993; Merrill et al., 1989] and has the ability to bind and produce homogeneous mixtures with acidic phospholipids (particularly PS), decreasing the cross-sectional area of the lipid head groups [Koiv et al., 1993]. Thus, a likely possibility is that the interaction of PS and sphingosine stimulates sphingosine kinase activity by neutralization of sphingosine charge [Koiv et al., 1993] or by changing the packing of the lipids [Koiv et al., 1993; Lopez-Garcia et al., 1993], which could result in an increase in the accesibility of sphingosine (its substrate) and/or PS (its potential activator) to sphingosine kinase. Interestingly, recent reports have demonstrated that sphingo-

sine increased PS production by activation of the L-serine base exchange enzyme in brain membranes [Kanfer and McCartney, 1991] and in LA-N-2 cells [Singh et al., 1992]. This enzyme appears to be the main pathway for PS production in mammalian cells [Kanfer and McCartney, 1991] by remodeling of preexisting molecules. Since sphingosine levels can be regulated in cells, we propose that an increase in sphingosine could induce lipid interactions with PS or other acidic phospholipids resulting in a recruitment of substrate or an activation of sphingosine kinase.

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