Journal of Cellular Biochemistry 55:146–153 (1994)

Naturally Occurring Ether-Linked Phosphatidylcholine Activates Phosphatidylinositol 3-Kinase and Stimulates Cell Growth

Siniti Misra, Amitabha Ghosh, and Lyuba Varticovski

Harvard School of Public Health, Boston, Massachusetts 02115 (S.M.); Bose Institute, Calcutta, India (A.G.); and Department of Biomedical Research, St. Elizabeth's Hospital, Boston, Massachusetts 02135 (S.M., L.V.)

Abstract Phosphatidylcholine (PC) from marine invertebrates is enriched in ether-linked forms. PCs from ray fish, *Dasyatis* sp., and bivalve, *Macoma birmanica,* used in the present study, contain 65% and 75% (w/w of total PC) of ether-linked forms, respectively. Ether-linked PCs also occur in mammalian membranes. Agonist-mediated hydrolysis of PC generates second messengers which participate in cellular responses. In this study, we tested whether PCs from marine invertebrates directly affect mammalian cell growth and activity of phosphatidylinositol (PI-3-kinase). PI-3-kinase participates in mitogenesis initiated by a variety of growth factors. PI-3-kinase converts polyphosphoinositides to 3′ phosphorylated isomers and these products accumulate in response to mitogenic stimuli. Whether cell membrane lipids regulate PI-3-kinase activity is not known. The marine animal–derived PCs and dioleoyl DAG (dioleoylglycerol) stimulated growth of murine pre-B lymphocytes, whereas chicken PC (egg lecithin) inhibited growth of these cells. Egg lecithin is also a potent inhibitor of PI-3-kinase activity in vitro. We studied the effect of PCs and DAG on PI-3-kinase activity. Unlike egg lecithin, marine animal PCs enhanced PI-3-kinase activity. We investigated the effect of lipids on PI-3-kinase substrate utilization. PCs enriched in ether-linked species increased utilization of substrates by PI-3-kinase. PCs purified from marine organisms also contain a substantially higher percentage of the cis-unsaturated fatty acids, especially of the $-\omega 3$ series (25% and 30% of total fatty acids for *Dasyatis* sp. and *Macoma birmanica,* respectively), as compared to vertebrate sources. In spite of differences in fatty acid composition, marine PCs and dioleoyl DAG showed similar effects on cell growth and PI-3-kinase activity. These findings indicate that ether-linked phospholipids activate PI-3-kinase and may participate in mitogenic responses.

Key words: cell growth, egg lecithin, ether-linked phospholipids, phosphatidylcholine, phosphatidylinositol, PI-3-kinase

Protein kinase C (PKC) takes part in cellular responses to various agonists including hormones, neurotransmitters, and growth factors. Most PKC isoenzymes are activated by diacylglycerol (DAG) [Nishizuka, 1992]. DAG is produced by hydrolysis of inositol phospholipids as part of an early cellular response. The hydrolysis of phosphatidylcholine (PC) produces DAG at a relatively later phase. The choline moiety of PC appears to be exchanged with free inositol to produce phosphatidylinositol (PI), which is phosphorylated by PI-4,5-kinase to phosphatidylinositiol 4,5-bisphosphate (PIP_2). The latter is subsequently hydrolyzed by PI-specific phospholipase C to produce DAG and inositol 1,4,5-trisphosphate (IP_3) [Nishizuka, 1992]. Therefore, DAG is a vital second messenger involved in signal transduction and cell growth.

We recently described novel ether-linked PCs and neutral lipids present in mammalian cells [Rana et al., 1991, 1992] and marine invertebrates [Chattopadhyay et al., in press]. These PCs and egg lecithin, which is similar to mammalian PC because it contains diacylglycerol PC, were equally sensitive to produce DAGs by action of phospholipase C (PLC). Metabolism of PCs enriched on ether-linked species leads to production of 1-o-alkenyl- and 1-o-alkyl-2-acylglycerol, which also could have significant effects on mammalian cell growth. Our study indicates that, in contrast to egg lecithin, PCs derived

Received November 18, 1993; accepted December 14, 1993.

Address reprint requests to Siniti Misra, Department of Biomedical Research, St. Elizabeth's Hospital, Boston, MA 02135.

from marine invertebrates potentiate IL-3–dependent cell growth of murine lymphocytes. Furthermore, PCs from marine origin stimulate PI-3-kinase, a novel phospholipid kinase [Whitman et al., 1988; Cantley et al., 1991] implicated in regulation of mitogenic responses. In contrast, egg lecithin, which is a potent inhibitor of PI-3-kinase activity [Carpenter et al., 1990], inhibited cell growth. The role of these lipids is discussed in relation to the regulation of PI-3-kinase activity and cell growth.

MATERIALS AND METHODS

Materials

All materials used for cell cultures were obtained from Gibco (Grand Island, NY). Lipid standards, phosphatidyl choline (PC),1,2-dioleoyl-*rac*-diacylglycerol (DAG), leupeptin, phenylmethylsulphonyl fluoride, aprotinin, adenosine 5′ triphosphate (ATP), and [γ^{32}P]-ATP (3 Ci/μ mol) were purchased from Dupont NEN (Boston, MA). Phospholipase C from *Bacilus aureus* was purchased from Sigma Chemical Company (St. Louis, MO). Solid supports and liquid phases for gas liquid chromatography (GLC) columns were obtained from Pierce Chemical (Rockford, IL). Silica gel G and H for thin-layer chromatography (TLC) were obtained from Merck (Dermstadt, FRG). Silicic acid used for column chromatography (Unisil 100–200 mesh) was from Clarkson Chemical (Williamsport, PA).

Buffers

Extraction buffer contained 25 mM Tris, pH 7.4, 10% glycerol, 1% Nonidet P-40 (NP-40), 50 mM NaF, 10 mM sodium pyrophosphate (PPi), 1 mM sodium vanadate (vanadate), protease inhibitors (leupeptin, pepstatin, and aprotinin at 10 μg/ml, and phenylmethyl-sulfonyl fluoride at 1 mM) [Whitman et al., 1988]. The PI-3-kinase assay buffer contained 25 mM 4-morpholinopropanesulfonic acid (MOPS), pH 7.0, 5 mM $MgCl_2$, and 1 mM EGTA as described by Susa et al. [1992]. The phospholipid sonication buffer contained 25 mM MOPS, pH 7.0, and 1 mM EGTA [Susa et al., 1992].

Collection of Samples

The two marine organisms—ray fish, *Dasyatis* sp., and bivalve organism, *Macoma birmanica*—were collected from Sagar Island, which is situated between lattitude 21° 31′ to 22° 30′ N and meridians 88° 08′ to 88° 16′ E. Individual animals were killed immediately after collection, livers were removed and stored at −25°C. Tissues from different collections were pooled and stored for lipid extraction.

Isolation and Analysis of Lipids

Lipids were extracted from the organs according to Bligh and Dyer [1959] and stored under nitrogen in redistilled hexane at −18°C until used. The total lipid was resolved into neutral-, glyco-, and phospholipids (PL) by column chromatography on silicic acid [Rouser et al., 1967]. Separation of PL and purification of phosphatidylcholine (PC) was carried out on silica gel H plates by one-dimensional TLC according to Hadjiagapiou and Spector [1987], using chloroform-methanol-40% methyl amine (60:20:5; v:v). These PCs contained three molecular species, plasmalogenic, alkylacyl-, and diacyl phospholipids. In order to determine the ratio of each species, the purified PCs were separated according to Rana et al. [1991]. Briefly, PC was run on silica gel H plates in chloroform/methanol/25% ammonia (65:25:5; v:v) according to Rouser et al. [1970]. The plates were exposed to HCl vapor for 4 min after development in the first direction, and nitrogen gas was flushed over the plate for 1 hr before the plates were developed in the second direction [Viswanathan et al., 1968] using chloroform/methanol/acetic acid/water (60:80:20:10; v:v). Aldehydes and 2 acyl-sn-glycero-3-phosphocholine derivatives were separated from the mixture of diacyl- and alkyl-acyl PC. Measurement of phosphate [Chen et al., 1956] content in the mixture of alkyl-acyl, diacyl PCs, and that of the lyso- forms represented the alkenyl-acyl PC content. The combined diacyl and alkyl-acyl forms of PC were dephosphorylated using bacterial PLC. The neutral lipid formed after PLC digestion was acetylated and separated by TLC using a solvent system of petroleum ether (40°–60°)/diethyl ether/acetic acid (90:10:1; v:v). The separated alkyl-acyl acetyl and diacyl-acetyl glycerols were recovered and fatty acids were analyzed by GLC. Peak identification and condition of analysis were according to Rana et al. [1991].

Cell Culture

Murine interleukin-3 (IL-3)–dependent pre-B lymphocytes (BaF3) [Palacios and Stenmetz, 1985] were grown in suspension culture in RPMI 1640 medium containing 10% fetal calf serum

(FCS) and 10% conditioned medium (WCM) from an IL-3–producing cell line, WEHI. Cell viability was determined by Trypan blue exclusion and cell count was performed in a manual hemocytometer. Cells were synchronized to quiescence for 16 hr and plated at 2–3 × 10^6 cells/ml in starvation medium (SM) which consisted of RPMI 1640, 1% bovine serum albumin (BSA), and 0.1% WCM. Under these conditions, cell viability was maintained at over 90% [Susa et al., 1992]. After 16 hr, cells were washed in RPMI 1040 supplemented with 1% BSA and 5% WCM and plated in 45 mm tissue culture dishes in the presence or absence of PLs as described in the text. All PLs were dissolved in DMSO and added directly to cell cultures. An aliquot of growing cells was removed at 24, 48, and 72 hr for analysis of cell viability and count.

PI-3-Kinase Assay

In vitro PI-3-kinase assay was performed using cell extracts from BaF3 cells as a source of enzyme as described [Susa et al., 1992]. Under the conditions of the assay, no PI-4, PI-4,5, DAG kinase, or PLC activities detected [Susa et al., 1992; S. Misra and L. Varticovski, unpublished observations]. Briefly, cell extracts were diluted to obtain 0.5 μg of protein/assay and < 0.01% of NP40. The reaction mixture contained sonicated phospholipids (equal amounts of PS, PI, and PI-4,5P_2) at a final concentration of 0.2 mg/ml [Auger et al., 1989; Varticovski et al., 1989]. Addition of PCs and DAGs was performed by replacing PS (used as a carrier) with the lipids of interest [Carpenter et al., 1990] as indicated in *Results*. The reaction was started by addition of [γ-^{32}P] ATP at a final concentration of 150 μM (4 mCi/nMol) in a total volume of 50 μl. Reaction was performed at 37°C for 20 min [Susa et al., 1992] and stopped by addition of methanol:1 N HCl (1:1). Phospholipids were extracted twice with the same volume of chloroform, separated by TLC in n-propanol:1 N acetic acid (65:35; v:v), and ^{32}P incorporation into PIP_3 was quantified by scintillation counting of corresponding spots from TLC [Auger et al., 1989; Varticovski et al., 1989]. To study whether the effect of marine PL on PI-3-kinase activity was due to altered presentation of substrates, PCs from *Dasyatis* sp. and *M. birmanica* were mixed with equal amounts of PS, dissolved in chloroform, evaporated under a stream of nitrogen, and the mixture resuspended in sonication buffer by ultrasonication for 10 min at 0.2 mg/ml. The mixed liposomes were added to PI-3-kinase assay after a 5 min preincubation with the standard PL mixture containing PS, PI, and PIP_2 (1:1:1, w/w) as above. The reaction was initiated by the addition of ATP. Enzymatic activity was expressed as pmoles of phosphate incorporated into PI-3,4,5P_3/min/mg of protein (units/ml; units/mg) as described [Susa et al., 1992].

RESULTS

Composition of PCs and Identification of Fatty Acids From *Dasyatis* sp. and *Macoma birmanica*

Analysis of PCs of *Dasyatis* sp. and *M. birmanica* revealed that PCs of *Dasyatis* sp. contained 44% plasmalogenic, 21% alkyl-acyl, and 35% diacyl PC. PCs from *M. birmanica* contained 35% plasmalogenic, 40% alkyl-acyl, and 25% diacyl PC (data not shown). The fatty acid composition of PCS obtained from the two organisms is presented in Table I. The palmitic acid (16:0) was the major component of all PCs. The next major saturated fatty acid was stearic acid (18:0). Total unsaturated fatty acid constituted 46% in *Dasyatis* sp. and 53% in *M. birmanica*. −ω3 polyunsaturated fatty acids were the major components. Among the −ω3 polyunsaturated fatty acids, eicosapentaenoic acid (20:5 ω3) was the major component in PCs from *M. birmanica* and docosahexaenoic acid (22:6 ω3) was the major component in *Dasyatis* sp.

Effect of IL-3, DMSO, and Ethanol on Cell Growth

To select appropriate PL solvent for the use in tissue culture, we tested DMSO and ethanol as carriers. Hematopoietic BaF3 cells grown in decreasing concentrations of IL-3 showed decreased cell viability when compared to control (Fig. 1). In 1:1 (5%, v/v) dilution of WCM in RPMI containing 1% BSA, over 90% of cells were viable at 24, 48, and 72 hr (Fig. 1). When cells were grown in increasing concentrations of DMSO, cell viability at 24 hr began to decrease in 0.25% (v/v) of DMSO. Using similar concentrations of ethanol in the same media, cell viability declined at 0.5% (v/v) ethanol (Table II). Therefore, we selected 0.25% DMSO and 5% WCM as the optimal conditions for further experiments.

Effect of PCs From *Dasyatis* sp., *Macoma birmanica*, Egg Lecithin, and DAG on BaF3 Cell Growth

To determine the effect of lipids on BaF3 cell growth, cells were induced to quiescence and

TABLE I. Fatty Acid Composition of PCs From *Dasyatis* sp. and *Macoma birmanica*

Composition of fatty acids (% w/w)	*Dasyatis* sp.	*Macoma birmanica*
14:0[a]	4.0	6.0
16:0	30.0	20.0
16:1 ω7[b]	8.0	6.0
18:0	12.0	15.0
18:1 ω9	7.0	9.0
18:2 ω6	3.0	2.0
18:3 ω3	5.0	6.0
20:4 ω6	5.0	4.0
20:5 ω3	8.0	12.0
22:5 ω6	3.0	2.0
22:5 ω3	2.0	4.0
22:6 ω3	10.0	8.0
Others[c]	2.0	6.0

[a]First figure denotes carbon number and second figure denotes number of double bonds.
[b]ω values signify number of carbon atoms from last double bond to the end CH_3 carbon atom.
[c]Others include 18:4 ω3, 20:1 ω9, 22:0 and 22:4 ω3.

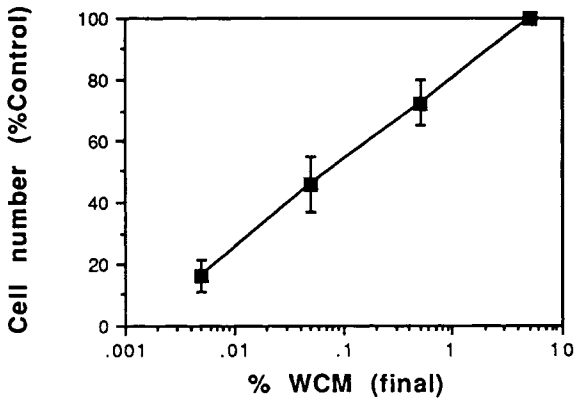

Fig. 1. The effect of IL-3 on BaF3 cell growth. Cells were induced to quiescence as indicated in *Materials and Methods* in the presence of 0.1% WCM. Under these conditions, cell viability was maintained at over 90%. After 16 hr, cells were washed in RPMI and plated in triplicate 45 mm tissue culture dishes with increasing concentrations of WCM. An aliqout of growing cells was removed for cell count. The results from two independent experiments are presented as the mean of cell number at 24 hr ±SE.

plated in RPMI, 1% BSA and 5% of WCM in increasing concentrations of PCs from *Dasyatis* sp., *Macoma birmanica,* egg lecithin, or dioleoylglycerol (DAG). Maximal effect was observed at 5 μg/ml. Higher concentrations were toxic to the cells. Control cells were grown in 0.25% DMSO. BaF3 cells grown in the presence of DAG or marine PCs showed an increase in cell number, whereas similar concentrations of egg

TABLE II. Effect of Phospholipid Carriers on Cell Growth*

Carrier content (% final)	Cell # (% C) DMSO	Ethanol
0	100	100
0.1	100	100
0.25	92	90
0.5	70	86
1.0	54	44

*Phospholipid solvents were tested for the effect on BaF3 cell growth. Quiescent cells were grown in triplicate dishes in the presence of 0–1% DMSO or ethanol. Cell counts were performed at the end of a 24 hr incubation in the presence of 5% WCM.

lecithin resulted in a decrease in cell number as compared to controls (Fig. 2A). Dioleoyl DAG was a potent stimulator of cell growth (Fig. 2B). Similar effects were observed when dimyristoyl DAG was used (not shown). The maximal effect on cell number in PC-treated cells was obtained at 48 hr. At 72 hr, cell number declined in all samples due to lack of IL-3. The above results suggest that marine organism PCs and DAGs potentiate IL-3–dependent BaF3 cell growth, whereas chicken PC inhibits cell growth.

Effect of PCs and DAGs on PI-3-Kinase Activity

We tested whether PCs from marine organisms, egg lecithin, and DAGs affect PI-3-kinase activity in vitro. PI-3-kinase activity was quantified by production of PI 3-P and PIP_3 from PI and PI-4,5P_2 in the presence and absence of PCs and DAGs [Varticovski et al., 1989]. Whole-cell lysates from BaF3 cells diluted over 1,000-fold to less than 0.001% of NP-40 and less than 1 μg of protein/assay [Susa et al., 1992; Susa and Varticovski, submitted] were used as a source of enzyme. The effect of various lipids on PI-3-kinase activity as measured by formation of PIP_3 is presented in Figure 3A and B. PCs from marine organisms stimulated PI-3-kinase activity at 0.08 and 0.1 mg/ml (Fig. 3A). The maximal increase in PI-3-kinase activity in the presence of 0.08 mg/ml of PCs from *Dasyatis* sp. and *M. birmanica* was 1.2- and 1.6-fold, respectively. Similar concentrations of DAG resulted in a threefold increase. Egg lecithin was a potent inhibitor of PI-3-kinase activity with a ½ max inhibition at 0.04 mg/ml (Fig. 3B), as also found by Carpenter et al. [1990]. These results correlate with the effect of these lipids on cell growth. Marine PCs which stimulated PI-3-kinase activity in vitro potentiated cell growth, whereas the

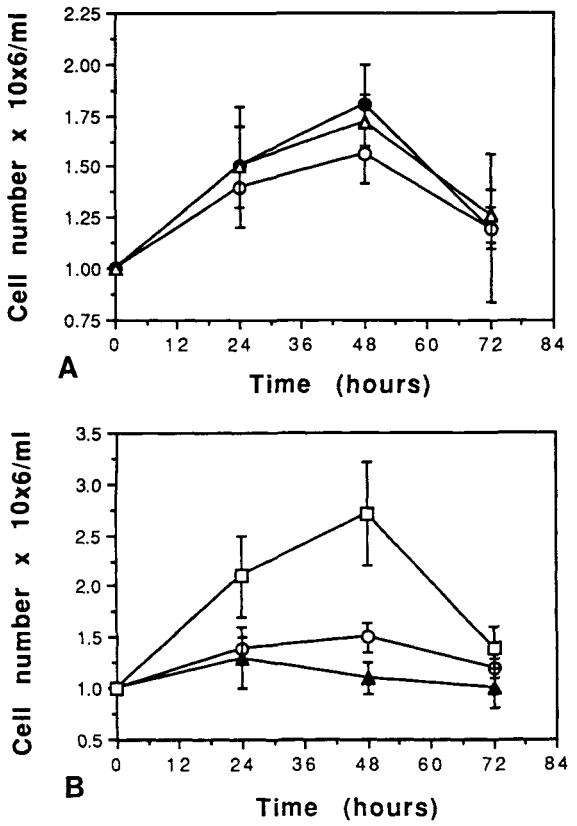

Fig. 2. **A:** Effect of PCs from *Dasyatis* sp. (closed circles) and *Macoma birmanica* (open triangles) on BaF3 cell growth. Cells were synchronized to quiescence for 16 hr and plated in triplicate samples in RPMI medium containing 1% BSA and 5% WCM in the presence or absence of 5 μg of PCs. Control cells were grown in the presence of carrier alone (0.25% DMSO) (open circles). The cells were counted at 24, 48, and 72 hr. The results of two independent experiments performed in triplicate dishes are presented ±SE. **B:** Effect of egg lecithin (closed triangles) and commercially available dioleoyl glycerol (DAG) (open squares) on cell growth. Control cells were grown in the presence of carrier and the cell number is indicated by open circles. Experimental conditions were same as in A. The results are presented ±SE from two independent experiments performed in triplicate samples.

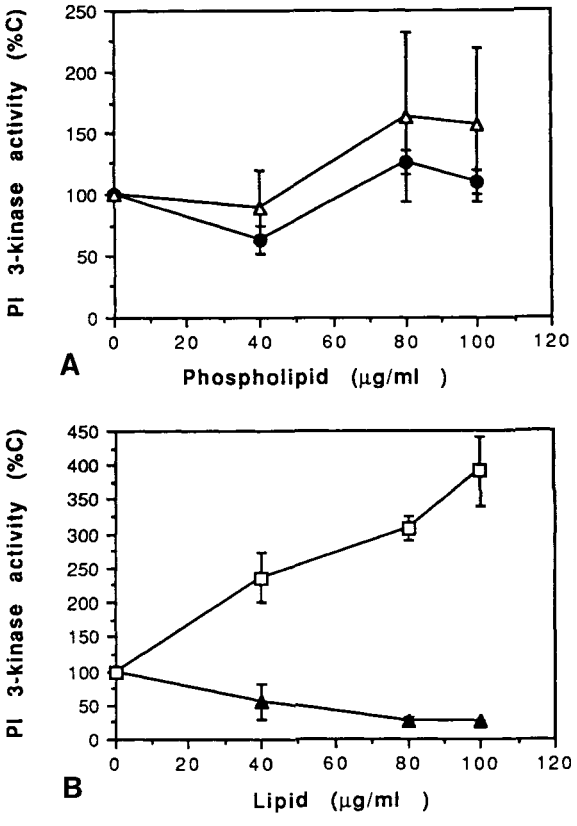

Fig. 3. **A:** Effect of marine invertebrate–derived PCs on PI-3-kinase activity. PCs from *Dasyatis* sp. (closed circles) and *Macoma birmanica* (open triangles) were sonicated at 0–100 μg/ml with the standard PI-3-kinase PL mixture (total PL of 200 μg/ml) as indicated in *Materials and Methods*. The reaction was started by the addition of γ^{32} P ATP and the production of PIP_3 was detected and quantified by TLC. The enzymatic activity was expressed as pmoles of phosphate incorporated into PI 3,4,5 P_2/min/mg of protein (units/ml; units/mg) [Susa and Varticovski, 1992]. Each experiment was performed on duplicate samples and the results of two independent experiments are presented ±SE. **B:** Effect of egg lecithin and dioleoyl glycerol (commercial DAG) on PI-3-kinase activity. The effect of egg lecithin is indicated as closed triangles and the effect of DAG is indicated as open squares. Experimental conditions were the same as in A. Each experiment was performed on duplicate samples and the results of two independent experiments are presented ±SE.

decrease in cell number induced by egg lecithin correlated with inhibition of PI-3-kinase activity.

Effect of Marine PCs on PI-3-Kinase Substrate Presentation

PI-3-kinase phosphorylates PI, PI-4-P, and PI-4,5P_2 in the inositol ring on position 3. Free inositols and other PLs, including PC, are not substrates for PI-3-kinase [Serunian et al., 1989]. To determine if changes in PI-3-kinase occur by altered substrate presentation, we measured PI-3-kinase activity in the presence of a mixture of two types of liposomes. Cell extracts containing PI-3-kinase were preincubated with the standard PL mixture. The lipids of interest were presented 5 min later, immediately prior to addition of ATP. The results presented in Figure 4A indicate that marine PCs, presented in separate liposomes, efficiently activate PI-3-kinase. Twofold activation occurred with a low concentration of marine PCs (0.04 μg/ml), and a maximal increase of threefold occurred at 0.08 μg/ml. Similar effects were observed with dioleoyl DAG

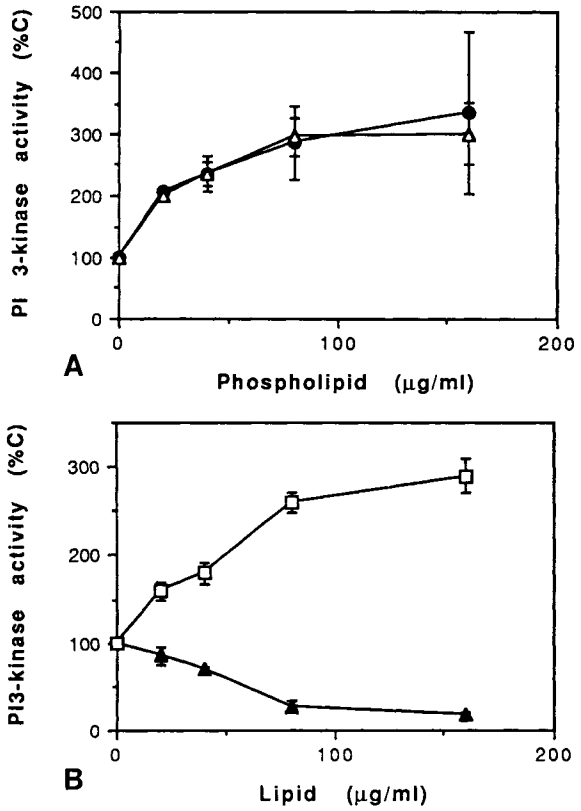

Fig. 4. A: Effect of marine invertebrate–derived PCs on PI-3-kinase substrate presentation. PCs from *Dasyatis* sp. (closed circles) and *M. birmanica* (open triangles) were mixed with equal amounts of PS to a total of 200 μg/ml and suspended in sonication buffer by ultrasonication for 10 min. The mixed liposomes were added to PI-3-kinase assay after a 5 min preincubation with standard substrates containing PS, PI, and PI-4,5-P2. The reaction was initiated by the addition of ATP. Enzymatic activity was expressed as described in Figure 3A. Each experiment was performed on duplicate samples and the results of two independent experiments are presented ±SE. **B:** Effect of egg lecithin (closed triangles) and commercially available dioleoyl glycerol (DAG) (open squares) on PI-3-kinase substrate presentation. Experimental conditions were the same as in A. Each experiment was performed on duplicate samples and the results of two independent experiments are presented ±SE.

(Fig. 4B) and myristoyl DAG (not shown). DAGs obtained by enzymatic hydrolysis of marine PCs also stimulated PI-3-kinase activity (not shown). In contrast, chicken PC (egg lecithin) was a potent inhibitor of PI-3-kinase activity in the same assay (Fig. 4B). These results indicate that changes in PI-3-kinase activity are most likely due to altered PI-3-kinase catalytic activity.

DISCUSSION

In this study, we tested whether PCs from marine invertebrates have a direct effect on cell growth. *Dasyatis* sp. and *M. birmanica* PCs contain 65% and 75% (w/w of PC) of ether-linked PCs, respectively. In contrast, vertebrate membranes primarily contain diacyl forms of PC. Marine animal–derived PCs, dioleoyl and dimyristoyl DAG, potentiated IL-3–induced growth of murine pre-B lymphocytes, BaF3. In contrast, chicken-derived PC was a potent inhibitor of cell growth. Agonist stimulation results in production of DAG through activation of phospholipase C [Kishimoto et al., 1980; Berridge, 1984; Nishizuka, 1984]. Accumulation of ether-linked species of DAG during mitogenic responses is thought to originate from hydrolysis of PC [Besterman et al., 1986; Daniel et al., 1986; Kennerly, 1987]. Naturally occurring ether-linked diacylglycerols, 1-O-alkyl-1′-enyl-2-acyl-sn-glycerol and 1-O-alkyl-2-acyl-sn-glycerol, also enhance PKC-mediated phosphorylation of histones and brain synaptosomal proteins [Ford et al., 1989]. Furthermore, Blitterswijk et al. [1987a] and Heesbeen et al. [1991] showed stimulation of PKC by ether-linked lipids. In contrast, other species of PC, such as 1-O-alkyl-2-O-methylglycero-3-phosphocholine, inhibit cell growth [Blitterswijk et al., 1987b; Noseda et al., 1988] in vitro and in vivo. The inhibitory effect of these PCs is likely to be mediated by generation of 1-O-alkyl-2-O-methylglycerol, which inhibits DAG-stimulated activity of purified human protein kinase C (PKC) and the binding of phorbol ester to PKC in HL-60 cells [Blitterswijk et al., 1987b; Noseda et al., 1988]. Therefore, marine PC-induced stimulation of cell growth could be, at least in part, due to generation of ether-linked species of DAGs and subsequent activation of PKC. To further understand the mechanism of action of marine PCs on cell growth, we investigated the effect of these lipids on PI-3-kinase, a key enzyme in mammalian mitogenic response.

We observed that PI-3-kinase activity was inhibited by diacyl PC and activated by similar concentrations of PCs which are enriched in ether-linked forms. Our results agree with the previously described inhibition of highly purified mammalian PI-3-kinase by egg lecithin [Carpenter et al., 1990]. We further tested whether the changes in PI-3-kinase activity were due to an altered presentation of substrates or interaction with the enzyme. PI-3-kinase activity was increased when standard substrates and marine animal PCs were added in separate liposomes. In contrast, egg lecithin presented to the enzyme in separate liposomes inhibited PI-3-

kinase activity. Commercially available DAGs or DAGs derived from marine animal PCs also stimulated PI-3-kinase activity. To achieve similar effects on PI-3-kinase activity, only a half of the concentration of PLs was required in this assay as compared to the previous experiments when PLs and the substrates were prepared in the same liposomes. These results suggest that ether-linked PCs and DAGs enhance utilization of PI-3-kinase substrates by direct interaction with the enzyme.

When compared to mammalian PC, marine PCs contain a higher percentage of cis-unsaturated fatty acids, particularly of the $-\omega 3$ series. Nishizuka [1992] showed that, in agonist-stimulated cells, PKC activity increases during the rise in intracellular Ca^{2+}. PKC activity is sustained after the intracellular Ca^{2+} declines providing that DAG and cis-unsaturated fatty acids are available. The high content of polyunsaturated fatty acids in marine PCs (25 and 30% of total for *Dasyatis* sp. and *Macoma birmanica,* respectively) could be biologically important. Marine PCs and commercial DAGs differ significantly because the former contain various chain lengths of polyunsaturated fatty acids, whereas DAGs, used in this study, contain only oleic or myristic fatty acids. In spite of the differences in fatty acid composition, marine PCs and DAGs had similar effects on cell growth and PI-3-kinase activity, suggesting that fatty acid composition is not critical for these biological activities.

The results of the present study demonstrate that marine but not chicken PCs support cell growth and activate PI-3-kinase. This is the first demonstration of activation of PI-3-kinase by naturally occurring lipids. Whether these lipids affect PI-3-kinase activity in intact cells requires further study.

ACKNOWLEDGMENTS

This work was supported in part by the National Fellowship by Department of Biotechnology, Government of India (S.M.), and NCI Grant R29CA530094 (L.V.).

REFERENCES

Auger KR, Serunian LA, Soltoff SP, Libby P, Cantley LC (1989): PDGF-dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. Cell 57:167–175.

Berridge MJ (1984): Inositol triphosphate and diacylglycerol as second messengers. Biochem J 220:345–360.

Besterman JM, Duronio V, Cuatrecasas P (1986): Rapid formation of diacyl glycerol from phosphatidylcholine: A pathway for generation of a second messenger. Proc Natl Acad Sci USA 83:6785–6789.

Bligh EG, Dyer WJ (1959): A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917.

Blitterswijk WJV, Bend RLV, Kramer IJM, Verhoeven AJ, Hilkmann H, Widf JD (1987a): A metabolite of an antineoplastic ether phospholipid may inhibit transmembrane signalling via protein kinase C. Lipids 22:842–846.

Blitterswijk WJV, Hilkmann H, Storme GA (1987b): Accumulation of an alkylysophospholipid in tumor cell membranes affects membrane fluidity and tumor cell invasion. Lipids 22:820–823.

Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S (1991): Oncogenes and signal transduction. Cell 64:281–302.

Carpenter CL, Duckworth BC, Auger KR, Cohen B, Schaffhausen BS, Cantley LC (1990): Purification and characterisation of phosphoinositide 3-kinase from rat liver. J Biol Chem 265:19704–19711.

Chattopadhyay G, Ghosh A, Choudhury A, Varticovski L, Dolnikowski GG, Misra S (1993): 1-o-alk-1′-enyl-2-acyl-sn-glycero-3-phosphocholines from five different tissues of the bivalve *Macoma birmanica.* Lipids, in press.

Chen PS, Toribara TY, Warner H (1956): Microdetermination of phosphorous. Anal Chem 28:1756–1758.

Christie WW (1982): In: "Lipid Analysis." 2nd ed. Oxford: Pergamon Press.

Daniel LW, Waite M, Wykle RL (1986): A novel mechanism of diglyceride formation. J Biol Chem 261:9128–9132.

Ford DA, Miyake R, Glaser PE, Gross RW (1989): Activation of protein kinase C by naturally occurring ether-linked diglycerides. J Biol Chem 264:13818–13824.

Hadjiagapiou C, Spector AA (1987): Docosahexaenoic acid metabolism and effect on prostacyclin production in endotheliai cells. Arch Biochem Biophys 253:1–12.

Heesbeen EC, Verdonck LF, Hermans SWG, Heugten HGV, Staal GEJ, Rijksen G (1991): Alkyllysophospholipid ET-18-OCH3 acts as an activator of protein kinase C in HL-60 cells. FEBS Lett 290:231–234.

Kennerly DA (1987): Diacylglycerol metabolism in Mast cell. J Biol Chem 262:16305–16313.

Kishimoto A, Takai Y, Mori T, Kikkawa U, Nishizuka Y (1980): Activation of calcium and phospholipid dependent protein kinase by diacylglycerol. Its possible relation to phosphatidylinositol turnover. J Biol Chem 255:2273–2276.

Nishizuka Y (1984): The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature 308:693–698.

Nishizuka Y (1992): Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614.

Noseda A, Godwin PL, Modest EJ (1988): Effects of antineoplastic ether lipids on model and biological membranes. Biochim Biophys Acta 945:92–100.

Palacios R, Stenmetz M (1985): IL-3 dependent mouse clones that express B-220 surface antigen, containing Ig genes in germ-line configuration, and generate B lymphocytes in vivo. Cell 41:727–734.

Rana APS, Majumdar GC, Misra S, Ghosh A (1991): Lipid changes of goat sperm plasma membrane during epididymal maturation. Biochim Biophys Acta 1061:185–196.

Rana APS, Majumdar GC, Misra S, Ghosh A (1992): Occurrence of wax esters and 1-0-alkyl-2,3-diacylglycerols in goat epididymal sperm plasma membrane. Lipids 27:75–78.

Rouser G, Kritchevsky G, Yamamoto A (1967): Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In Marinetti GV (ed): "Lipid Chromatographic Analysis." Vol 1. New York: Mercel Dekker, pp 99–162.

Rouser G, Fleischer S, Yamamoto A (1970): Two dimensional thinlayer chromatographic separation of polar lipids and determination of phospholipids by phosphorous analysis of spots. Lipids 5:494–496.

Serunian LA, Haber MT, Fukui T (1989): Phosphoinositides produced by phosphatidylinositol 3-kinase are poor substrates for phospholipase C from rat liver and bovine brain. J Cell Biol 264:17809–17815.

Susa M, Varticovski L (1993): Expression of BCR-abl activates phosphatidylinositol 3-kinase in hematopoietic cells but not in NIH 3T3 fibroblasts. J Biol Chem, submitted.

Susa M, Keeler M, Varticovski L (1992): Platelet-derived growth factor activates membrane-associated phosphatidylinositol 3-kinase and mediates its translocation from the cytosol. J Biol Chem 267:22951–22956.

Varticovski L, Drucker B, Morrison LC, Cantley LC, Roberts T (1989): The colony stimulating factor-1 receptor associates with and activates PI 3-kinase. Nature 342:699–702.

Viswanathan CV, Phillips F, Lundberg WO (1968): J Chromatogr 35:66–71.

Whitman M, Downes CP, Keeler M, Keeler T, Cantey L (1988): Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. Nature 332:644–646.