Synthetic Branched-Chain Analogues of Distearoylphosphatidylcholine: Structure–Activity Relationship in Inhibiting and Activating Protein Kinase C†

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ABSTRACT: A series of distearoylphosphatidylcholine (DSPC) analogues having various branched alkyl chains were synthesized and tested for their abilities to regulate protein kinase C (PKC). The greatest improvement (about 3-fold) in the PKC inhibitory activity over that seen for the parental lipid (i.e., DSPC) was accomplished by substitution of 8-methylstearate at *sn*-2 and 16-methylstearate at both *sn*-1 and *sn*-2 positions of glycerol; substitutions at both *sn*-1 and *sn*-2 with 8-methylstearate, on the other hand, caused a decrease (about 4-fold) in its inhibitory activity. Introduction of butyl, phenyl, or keto functions to various positions in the fatty alkyl chain substituted at both *sn*-1- and *sn*-2 positions imparted upon the DSPC analogues an ability to potently stimulate PKC to an extent comparable to those attainable by diacylglycerol or phorbol ester; the analogues having substitution only at the *sn*-2 position, in comparison, had no or reduced stimulatory activity. The butyl, phenyl, and keto analogues of DSPC, as with DSPC itself and its methyl analogues, inhibited PKC at high concentrations. Kinetic analysis indicated that the methyl DSPC analogues inhibited the enzyme competitively with respect to phosphatidylserine (PS; a phospholipid cofactor) and Ca^{2+}. The butyl analogues activated the enzyme without affecting its affinity for PS or Ca^{2+}, indicating a mechanism different from that seen for diacylglycerol or phorbol ester. The inhibitory activity of the methyl DSPC analogues and the stimulatory activity of the butyl DSPC analogues were reduced when PKC was activated by phorbol ester. Both classes of the analogues were unable to compete for the binding of [^{3}H]phorbol dibutyrate to PKC. The findings suggest that DSPC analogues having different branched-chain fatty alkyl chains exhibited altered abilities to regulate PKC, probably reflecting in part some unique physicochemical properties of the analogues capable of critically modifying the activation process of the PKC system.

Protein kinase C (PKC)[1] is a phospholipid/Ca^{2+}-dependent enzyme shown to be involved in a number of cellular activities including transmembrane signaling, growth, and differentiation (Nishizuka, 1984). Of the naturally occurring phospholipids, PS is the most effective cofactor for the enzyme, whereas others, such as PC, are inhibitors of the enzyme (Takai et al., 1979; Kaibuchi et al., 1981). In addition, certain lipid substances including diacylglycerol (Kishimoto et al., 1980), unsaturated fatty acids (McPhail et al., 1984; Murakami et al., 1986), and lysophospholipids (K. Oishi, R. L. Raynor, P. A. Charp, and J. F. Kuo, unpublished data) are activators; ALP (Helfman et al., 1983), lipoidal amine CP46,665-1 (Shoji et al., 1985), sphingosine (Hunnun et al., 1986), and lysosphingolipids (Hunnun & Bell, 1987), on the other hand, are inhibitors. Because of a central role played by PKC in biological processes, elucidation of mechanisms underlying its inhibition and activation by various agents has been a subject of intensive investigation. Studies addressing the structure–activity correlates of diacylglycerols as PKC activators have appeared (Mori et al., 1982; Ganong et al., 1986). In the present studies, we synthesized a number of branched-chain analogues of DSPC and examined their effects on PKC. We found that certain analogues were relatively potent inhibitors or activators of the enzyme, depending upon the kinds and positions of substitutions in the fatty alkyl chains and the positions of the stearate derivatives in the glycerol backbone.

Experimental Procedures

Materials. The L-α-glycerophosphorylcholine–cadmium chloride complex and monostearoyllecithin (natural configuration) were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL); 1,16-hexadecanedioic acid was from Fluka Chemical Corp. (Ronkonkoma, NY); Dragendorff's reagent, PS, histone H1, and PDBu were from Sigma Chemical Co. (St. Louis, MO); Silicar silica gel (100–200 mesh) was from Mallinckrodt (St. Louis, MO); thin-layer chromatography sheets, precoated with a 254-nm fluorescent indicator, and all other starting materials and reagents for chemical synthesis were from Aldrich Chemical Co. (Milwaukee, WI); [^{3}H]PDBu (15.8 Ci/mmol) was from Du Pont/New England Nuclear (Albany, MA); TPA was from LC Services Corp. (Woburn, MA.); polymyxin B sulfate was from Upjohn Co. (Kalamazoo, MI).

Synthesis and Characterization of Branched-Chain Derivatives of DSPC. Since the synthesis of all the branched stearic

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[1] Abbreviations: PKC, protein kinase C; PS, phosphatidylserine; PC, phosphatidylcholine; ALP, alkyllysophospholipid; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; PDBu, phorbol 12,13-dibutyrate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; IC_{50}, concentration required for 50% inhibition; EC_{50}, concentration required for 50% effect (stimulation). The methyl (Me), butyl (Bu), phenyl (Ph), and keto analogues of branched-chain distearoylphosphatidylcholine (DSPC) were abbreviated as follows: For example, 2(8-Me)DSPC or 1,2(8-Me)DSPC indicated that the stearoyl moieties in DSPC were substituted by 8-methylstearic acid at only *sn*-2 or both *sn*-1 and *sn*-2 positions in the glycerol backbone (see Scheme II for structures).

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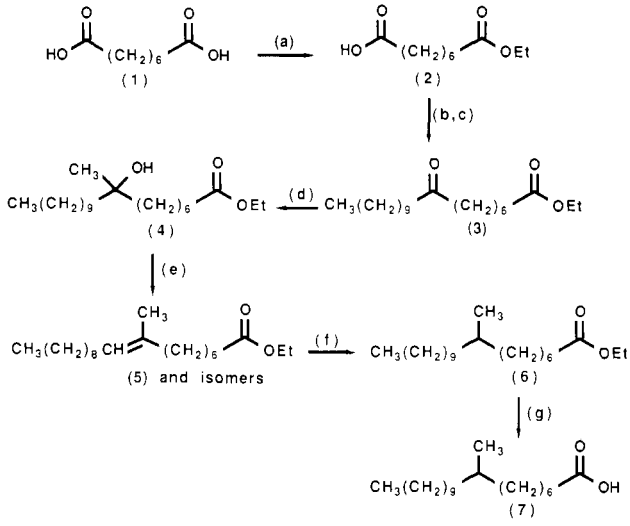
Scheme I: Synthesis of *dl*-8-Methylstearic Acid[a]

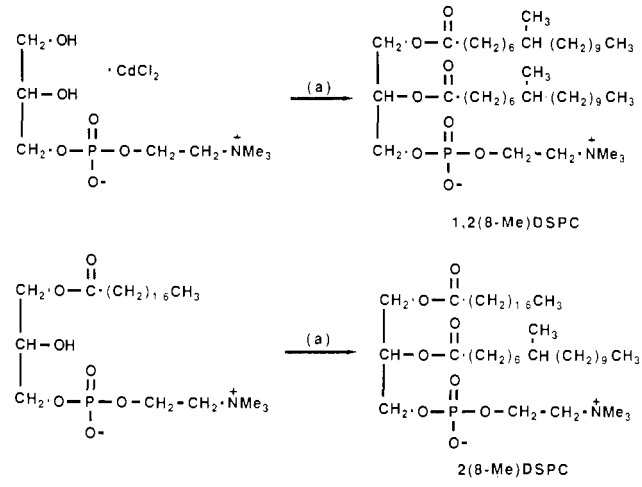


[a] Reaction conditions for the various steps were as follows: (a) ethanol/H_2SO_4/cyclohexane; (b) $SOCl_2$/heat; (c) $[CH_3(CH_2)_9]_2Cd$/benzene; (d) CH_3MgBr/ether; (e) PBr_3/pyridine/heat; (g) KOH/H_2O.

acid derivatives (30–50% overall yield) followed the same outline (Scheme I) (Cason et al., 1949), only one particular example will be given here. The monoester **2** was made by continuously extracting a mixture of diacid **1**, concentrated sulfuric acid, water, and ethanol with cyclohexane (Babler & Moy, 1979). The keto ester **3** was prepared by the reaction between the acid chloride of **2** and didecylcadmium (obtained via the Grignard of 1-bromodecane) in benzene (Cason, 1947; Cason & Prout, 1948; Shirley, 1949). Coupling of Grignard reagents, especially when the chains were 10 or 14 carbon atoms long, occurred as an undesirable side reaction. When the hydrocarbons formed, removal from the corresponding keto esters was effected by column chromatography (8:2 petroleum ether/diethyl ether). The keto ester **3** was then reacted with methylmagnesium bromide in ether at 0 °C and the resultant mixture subsequently stirred at room temperature for 4 h (Bovey & Turner, 1951). After dehydration of the alcohol **4** with PBr_3 and pyridine (Samokhvalov et al., 1956), the alkenes **5** (which were a mixture of isomers) were hydrogenated over PtO_2 under Parr conditions. Purified **6** was then hydrolyzed in aqueous KOH in excellent yield to give **7**. The methyl and ketone branched fatty acids were purified by recrystallization from acetone; the phenyl and butyl branched fatty acids were purified by column chromatography (7:3 petroleum ether/diethyl ether). All fatty acids were characterized by elemental analysis, high-resolution mass spectrometry, ^{13}C NMR, and proton NMR. The phospholipid synthesis (Selinger & Lapidot, 1966; Regan et al., 1982; Samuel et al., 1985), which gave 70–90% yields, is shown in Scheme II.

The DSPC derivatives were purified by silica gel chromatography, and their purity was determined by Dragendorff's reagent on thin-layer chromatograms. In all cases, single spots were observed for each of the derivatives, confirming their purity. Characterization of the DSPC derivatives was accomplsihed by fast atom bombardment mass spectrometry, ^{13}C NMR, and proton NMR. Their purity was further verified by thin-layer chromatography, monolayer experiments, and differential scanning calorimetry measurements. Proton NMR spectra were obtained on a Nicolet FT 360-MHz spectrometer or a General Electric QE 300-MHz spectrometer. ^{13}C NMR decoupled spectra were obtained with the General Electric QE 300-MHz spectrometer or with a Bruker WP-

Scheme II: Synthesis of 1,2(8-Me)DSPC and 2(8-Me)DSPC[a]



[a] Reaction conditions (a): 8-methylstearic acid/1,3-dicyclohexylcarbodiimide/4-(dimethylamino)pyridine/$CHCl_3$.

200SY spectrometer. High-resolution mass spectra and fast atom bombardment mass spectra were obtained on a VG Analytical Instruments MM-7070S mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. All analytical data were consistent with the expected structures of the branched-chain derivatives of DSPC.

PKC Assays and [^3H]PDBu Binding. PKC was purified through the phenyl-Sepharose step (Wise et al., 1982). The enzyme activity was assayed under standard conditions as described elsewhere (Girard et al., 1985). Briefly, the reaction mixture (0.2 mL) contained 5 μmol of Tris-HCl, pH 7.5, 2 μmol of $MgCl_2$, 2 μg of PS, 40 μg of histone H1, 0.06 μmol of EGTA, 1.6 nmol of [γ-^{32}P]ATP (containing $\sim 1.5 \times 10^6$ cpm), with or without 0.1 μmol of $CaCl_2$, and various kinds and concentrations of DSPC derivatives, as indicated. When present, the final concentration of $CaCl_2$ was 200 μM (in excess of 300 μM EGTA). The reaction was carried out at 30 °C for 5 min. In experiments (Figures 2 and 4) that utilized TPA to stimulate PKC, the standard assay conditions were modified to include TPA at a final concentration of 50 nM, and $CaCl_2$ was reduced to 10 μM. The DSPC derivatives were mixed with PS in chloroform/methanol (1:1) at the desired concentrations and dried in a stream of N_2. The dried lipids were resuspended in 20 mM Tris-HCl, pH 7.5, vortexed, and then sonicated by using a sonifier cell disruptor (Heat Systems-Ultrasonics Inc.) with a microtip (Plainview, NY), set at 60% power output for 30 s at 23 °C. The sonicated preparations of PS–DSPC analogues were clear at all concentrations and combinations of the lipids used. The binding of [^{3}H]PDBu to PKC was carried out by the procedure of Sharkey and Blumberg (1985), but using a low concentration (8 μg/mL instead of 100 μg/mL) of PS as we reported recently (Charp et al., 1988). All experiments were performed in duplicate or triplicate and were repeated 3–5 times to ascertain the reproducibility of the findings. [γ-^{32}P]ATP was prepared as described by Post and Sen (1967).

Results

DSPC inhibited PKC with an IC_{50} of 20 μM (Figure 1). When the stearic acid moieties at *sn*-2 (left panel) or both *sn*-1 and *sn*-2 positions of glycerol (right panel) possessed methylated constituents, the resulting DSPC analogues exhibited altered inhibitory potencies. Their IC_{50} values estimated from several experiments similar to those shown in Figure 1 are summarized (Table I). It was found that 1,2(16-M), 2(8-Me),

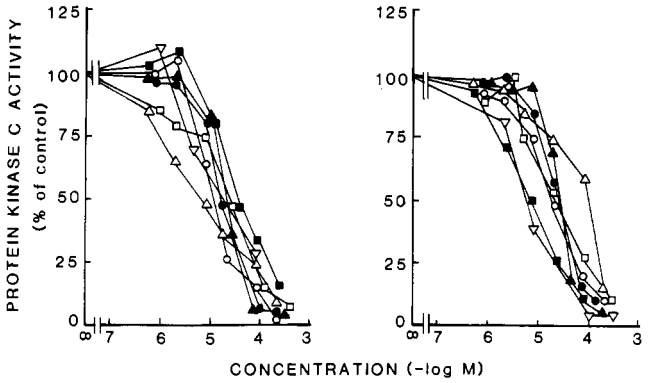


FIGURE 1: Inhibition of PKC by monomethyl and dimethyl analogues of DSPC and ALP. The enzyme (0.54 μg) was assayed under the standard conditions with 10 μg/mL PS (12.7 μM, assuming dioleoyl-PS) and 100 μM $CaCl_2$. The Ca^{2+}-stimulated enzyme activity seen in the absence of DSPC derivatives (16.2 pmol of P/min) was taken as 100%. The lipids had no effect on the basal activity on the enzyme, which amounted to less than 5% of the stimulated activity. See Experimental Procedures for further details. Left panel (DSPC analogues): (○) 2(4-Me); (●) 2(6-Me); (△) 2(8-Me); (▲) 2(10-Me); (□) 2(12-Me); (■) 2(16-Me). (▽) parental DSPC. Right panel (DSPC analogues): (○) 1,2(4-Me); (●) 1,2(6-Me); (△) 1,2(8-Me); (▲) 1,2(10-Me); (□) 1,2(12-Me); (■) 1,2(16-Me). (▽) ALP.

Table I: PKC Inhibition by DSPC and Its Methyl Analogues

compound	IC_{50}[a] (μM)	compound	IC_{50}[a] (μM)
DSPC	20 ± 2	1,2(4-Me)DSPC	17 ± 1
2(4-Me)DSPC	10 ± 1	1,2(6-Me)DSPC	30 ± 3
2(6-Me)DSPC	17 ± 1	1,2(8-Me)DSPC	91 ± 4
2(8-Me)DSPC	6 ± 1	1,2(10-Me)DSPC	37 ± 2
2(10-Me)DSPC	12 ± 2	1,2(12-Me)DSPC	20 ± 1
2(12-Me)DSPC	17 ± 3	1,2(16-Me)DSPC	6 ± 2
2(16-Me)DSPC	18 ± 2	ALP	7 ± 2

[a] Mean ± SE of three experiments.

and 2(4-Me) derivatives of DSPC were most active (IC_{50} values of 6, 6, and 10 μM, respectively). It seemed worth noting that 1,2(8-Me)DSPC (IC_{50} 91 μM) was the least active, compared with the most active 2(8-Me)DSPC (IC_{50} 6 μM), indicating that substitutions of 8-methylstearate at both *sn*-1 and *sn*-2 positions in glycerol had an effect opposite to that of substitution at only the *sn*-2 position. On the other hand, 1,2(16-Me)DSPC (IC_{50} 6 μM) was more potent than 2(16-Me)DSPC (IC_{50} 18 μM), further suggesting that the positions of the methyl function in the fatty alkyl chain could also influence the inhibitory activity of the analogues. ALP, an experimental antineoplastic agent shown previously to be a PKC inhibitor (Helfman et al., 1983) and included here for comparison, had an IC_{50} of 7 μM under the same experimental conditions (Figure 1; Table I).

It has been reported that TPA activated PKC by increasing its affinity for Ca^{2+} (Castagna et al., 1983). In order to maximize the TPA stimulatory effect, PKC was assayed under the modified condition with a low $CaCl_2$ concentration of 10 μM, compared with 200 μM under standard conditions as shown in Figure 1. Under this suboptimal condition for PKC activation, both 2(8-Me)- and 1,2(16-Me)DSPC inhibited the enzyme with IC_{50} values of 5–8 μM (Figure 2), similar to those seen under the standard condition shown earlier in Figure 1. The inhibitory potencies of the derivatives, however, were markedly decreased when PKC was further activated by 50 nM TPA, with IC_{50} values of about 100 and 500 μM for 2(8-Me) and 1,2(16-Me)DSPC, respectively (Figure 2).

In contrast to the strictly inhibitory effect of methyl-DSPC analogues shown above, the effects of the butyl analogues were biphasic; i.e., they stimulated the enzyme (160–330% of

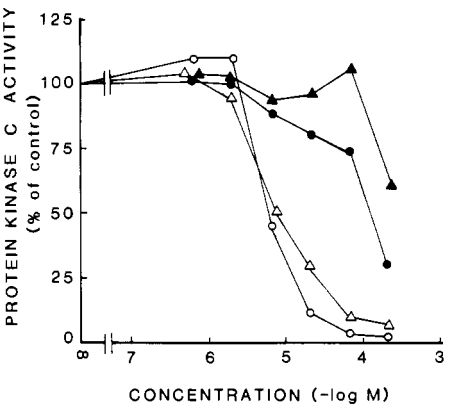


FIGURE 2: Comparative potency of methyl analogues of DSPC on PKC assayed in the absence or presence of TPA. The enzyme (0.54 μg) was assayed under the modified conditions (10 μg/mL PS and 10 μM $CaCl_2$). The enzyme activity values seen in the absence (2.3 pmol of P/min) and presence (7.8 pmol/min) of 50 nM TPA were taken as 100%, respectively. 2(8-Me)DSPC in the absence (○) or presence (●) of TPA; 1,2(16-Me)DSPC in the absence (△) or presence (▲) of TPA.

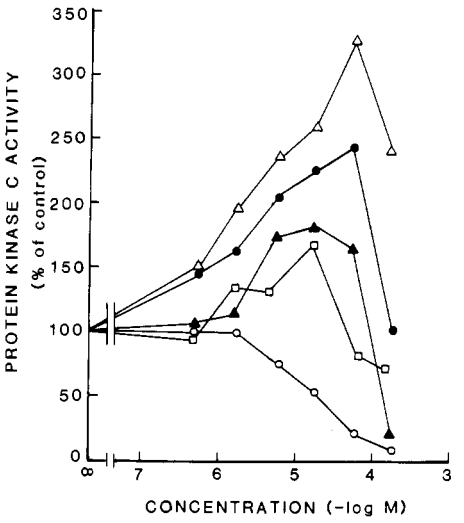


FIGURE 3: Stimulation and inhibition of PKC by monobutyl and dibutyl analogues of DSPC. The enzyme was assayed under the standard conditions as indicated in Figure 1. The Ca^{2+}-stimulated PKC activity seen in the absence of the analogues (15.7 pmol/min) was taken as 100%. The analogues had little or no effect on the basal activity of the enzyme seen in the absence of PS and $CaCl_2$. DSPC analogues: (○) 2(12-Bu); (●) 1,2(4-Bu); (△) 1,2(8-Bu); (▲) 1,2-(10-Bu); (□) 1,2(12-Bu).

control) at low concentrations while inhibiting it at high concentrations (Figure 3). It was further noted that the presence of butylated stearic acid moieties at both *sn*-1 and *sn*-2 positions of glycerol appeared to be required for the stimulatory effect, because the 1,2(4-Bu), 1,2(8-Bu), 1,2(10-Bu), and 1,2(12-Bu) analogues were stimulatory, whereas the 2(12-Bu) analogue was strictly inhibitory. The EC_{50} (2–3 μM) and IC_{50} (20–100 μM) values for the butyl analogues as well as the extent of their stimulation of PKC estimated from several experiments similar to those shown in Figure 3 are summarized (Table II). It should be emphasized here that the stimulatory effect of the analogues was observed with the standard assay condition (i.e., 200 μM $CaCl_2$) under which PKC was already nearly maximally activated and, furthermore, the extent of stimulation was comparable with that obtained with diacylglycerol or TPA (data not shown).

When assayed under the suboptimal condition (i.e., 10 μM $CaCl_2$), 1,2(8-Bu)DSPC also stimulated (230% of control) and inhibited PKC, with EC_{50} and IC_{50} values of 2 and 30 μM,

Table II: Stimulation and Inhibition of PKC by Butyl, Phenyl, and Keto Analogues of DSPC

compound	max stimulation[a] (% control)	EC_{50}[a] (μM)	stimulation/EC_{50}	IC_{50}[a,b]
2(12-Bu)DSPC	0			20.3 ± 1.8
1,2(4-Bu)DSPC	240 ± 20	3.1 ± 1.2	80	90.6 ± 3.2
1,2(8-Bu)DSPC	330 ± 28	3.2 ± 0.8	110	≈300
1,2(10-Bu)DSPC	180 ± 10	2.1 ± 0.2	90	80.8 ± 8.2
1,2(12-Bu)DSPC	160 ± 12	1.9 ± 0.2	80	20.6 ± 3.6
2(8-Ph)DSPC	150 ± 22	2.3 ± 1.1	75	20.9 ± 2.9
2(12-Ph)DSPC	140 ± 21	2.4 ± 0.2	70	9.0 ± 1.6
1,2(12-Ph)DSPC	200 ± 11	2.1 ± 0.3	100	45.6 ± 3.2
1,2(6-keto)DSPC	190 ± 12	25.6 ± 3.5	8	<1000

[a] Mean ± SE of three of five experiments. [b] Based on 50% inhibition of maximal activity.

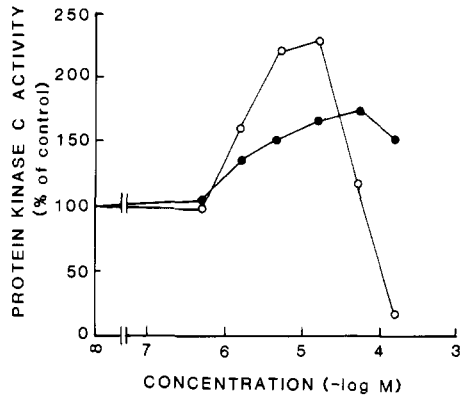


FIGURE 4: Comparative potency of 1,2(8-Bu)DSPC on PKC assayed in the absence or presence of TPA. The enzyme was assayed under the modified conditions as indicated in Figure 2. The enzyme activity values seen in the absence (○) or presence (●) of 50 nM TPA were taken as 100%, respectively.

respectively (Figure 4). The analogue further stimulated (170% of control) the TPA-activated enzyme, with a similar EC_{50} of 3 μM. The TPA-activated enzyme, in comparison, was much more resistant to inhibition by the analogue, with an estimated IC_{50} of >200 μM (Figure 4). The finding was similar to that made with the methyl analogues in that they were also much less effective in inhibiting PKC in the presence of TPA, as shown in Figure 2.

In order to further explore the effects of the nature and position of the substitutions in the branched-chain stearic acid moieties, additional but limited numbers of DSPC analogues were synthesized. It was found that 2(8-Ph)-, 2(12-Ph)-, and 1,2(12-Ph)DSPC stimulated and inhibited PKC (Figure 5) in a manner similar to those seen for the butyl analogues shown in Figure 3. Interestingly, 1,2(6-keto)DSPC was less potent in both activating and inhibiting the enzyme (Figure 5). The kinetic data of these analogues are also summarized and compared with those obtained for the butyl analogues (Table II). Judging from the ratios of maximal stimulation/EC_{50}, 1,2(6-keto)DSPC (with a value of 8) was the least effective activator of PKC compared with the butyl and phenyl analogues, with an exception of 2(12-Bu)DSPC, which was not an activator (Table II).

2(8-Me)DSPC and 1,2(8-Bu)DSPC, the two analogues found to be the most active inhibitor and activator of PKC, respectively, were used for studying their modes of action. Inhibition of the enzyme by the methyl analogue, characterized by an increased K_m for PS from 5 to 20 μg/mL, was overcome by increasing concentrations of PS, whereas the butyl analogue increased the V_{max} without affecting the K_m for PS (Figure 6, left panel). Similarly, the methyl analogue increased the

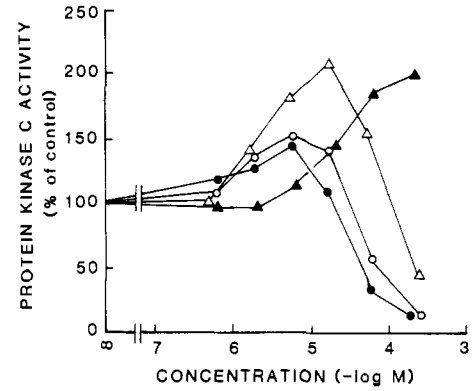


FIGURE 5: Stimulation and inhibition of PKC by phenyl and keto analogues of DSPC. The enzyme was assayed under the standard conditions as indicated in Figure 1. DSPC analogues: (○) 2(8-Ph); (●) 2(12-Ph); (△) 1,2(12-Ph); (▲) 1,2(6-keto).

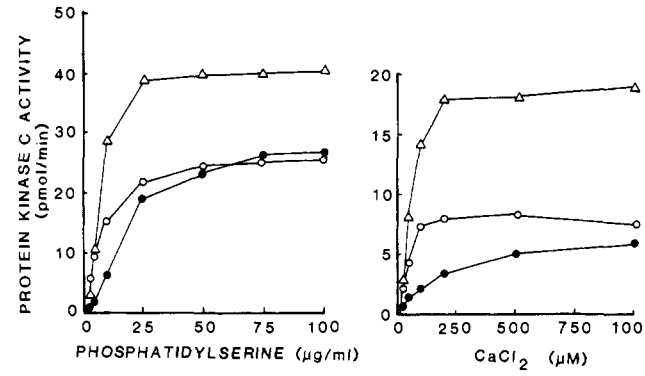


FIGURE 6: Kinetics of PKC inhibition and activation by DSPC analogues. The enzyme was assayed under the standard conditions in the absence (○) or presence of 12.4 μM 2(8-Me)DSPC (●) or 55.4 μM 1,2(8-Bu)DSPC (△) and in the presence of varying concentrations of PS (left) or $CaCl_2$ (right), as indicated.

K_a for $CaCl_2$ from 50 to 350 μM with its PKC inhibition being overcome by increasing concentrations of $CaCl_2$, whereas the butyl analogue increased the V_{max} without affecting the K_a for $CaCl_2$ (Figure 6, right panel). Similar kinetic findings were made with other methyl and butyl analogues of DSPC (data not shown).

Finally, we examined the effects of several DSPC analogues on the binding of [^{3}H]PDBu to PKC compared with that of polymyxin B, a specific inhibitor of PKC (Mazzei et al., 1982). As shown recently (Charp et al., 1988), polymyxin B competed with the binding with an IC_{50} of about 30 μM (figure not shown). In the present studies, we noted that the 2(8-Me), 1,2(16-Me), 1,2(4-Bu), and 1,2(8-Bu) analogues of DSPC could not inhibit phorbol ester binding at concentrations up to 300 μM (figure not shown). On the contrary, a slight increase in the binding was noted in the presence of high concentrations (>200 μM) of the analogues.

Discussion

We showed in the present studies a structure–activity relationship of the branched-chain analogues of DSPC in inhibiting or activating PKC. In a series of DSPC analogues having the methylated stearic acids substituted at *sn*-2 of glycerol, 2(8-Me)DSPC was found to be the most potent, indicating that the methyl group at the center of the alkyl chain (i.e., carbon 8) was critical for the inhibitory activity. In another series of analogues having the methyl fatty acid moieties at both *sn*-1 and *sn*-2 positions, 1,2(8-Me)DSPC was the least potent whereas 1,2(16-Me)DSPC was the most potent. These results clearly indicated that the position of the

methyl function in the alkyl chain and the position of branched-chain fatty acids in the glycerol backbone were important determinants. Although the improvement of the inhibitory potency afforded by 2(8-Me)DSPC and 1,2(16-Me)DSPC (IC_{50} for both 6 μM) over the parent compound (IC_{50} 20 μM) was quite modest, the analogues nonetheless were at least as potent as ALP (Helfman et al., 1983) and sphingosine (Hannun et al., 1986), the two best lipid inhibitors of PKC known to date.

It was surprising that butyl and phenyl analogues of DSPC at low concentrations could conversely activate PKC (EC_{50} 2–3 μM) to the extents comparable to those attainable by diacylglycerol and TPA. Butyl and phenyl groups are more bulky than the methyl group and hence would increase the hydrophobicity of the compounds. It appeared that this physical property might not be the sole factor responsible for their stimulatory activity, because 1,2(6-keto)DSPC, presumably less hydrophobic than the methyl analogues, was also stimulatory. The keto analogue, however, was a less potent activator of PKC than the butyl and phenyl analogues. Substitutions at both *sn*-1 and *sn*-2 of glycerol seemed to be important for the stimulatory activity, because 1,2(12-Bu)-DSPC and 1,2(12-Ph)DSPC were more active than the corresponding 2(12-Bu) and 2(12-Ph) analogues. It is of interest that 1,2(8-Bu)DSPC (IC_{50} ~300 μM) and 1,2(8-Me)DSPC (IC_{50} 91 μM) were the poorest inhibitors among their respective groups of analogues. The data suggested that a substitution at the center of the alkyl chain (i.e., carbon 8) at *sn*-1 and *sn*-2 would markedly decrease the inhibitory activity. The observation that 1,2(8-Bu)DSPC was more effective in activating PKC (330% of the control) than other butyl analogues seemed to be consistent with this notion.

Inhibition of PKC by 2(8-Me)DSPC was competitive with respect to PS and Ca^{2+}, suggesting that the methyl analogue interacted with the free enzyme form as did PS and Ca^{2+}. The binding sites of the analogue appeared to be distinct from those of phorbol esters, because it failed to compete for the binding of [^{3}H]PDBu to PKC. The enzyme activation by 1,2(8-Bu)DSPC was not involved in changes in the affinity of the enzyme for PS and Ca^{2+}. Its mode of activation, therefore, was different from that of diacylglycerol (Kishimoto et al., 1980) or TPA (Castagna et al., 1982) in that the latter two activators increased the affinity for PS and Ca^{2+}. The ability of the butyl analogue to further augment the TPA-stimulated PKC activity and its inability to replace [^{3}H]PDBu bound to the PKC/PS/Ca^{2+} complex further supported the conclusion.

Although the exact mechanisms by which the DSPC analogues regulate PKC remain unclear, multipoint but specific interactions of the analogues with the PKC/PS/Ca^{2+} or PKC/PS/Ca^{2+}/TPA complex are likely to occur. Preliminary results obtained from the monolayer experiments (F. M. Menger et al., unpublished results) suggested the existence of stearic acid chain distortion upon substitution with either butyl or phenyl groups, a phenomenon that was less noticeable with methyl substitution. This distortion or chain bending might be similar to that observed for the double-bond-containing fatty acids such as oleic acid or arachidonic acid, both of which were activators of PKC even in the absence of PS and/or Ca^{2+} (McPhail et al., 1984; Murakami et al., 1986). This principle might be also applicable to diacylglycerols containing long-chain fatty acids, because diolein is effective whereas distearin is a poor PKC activator (Kishimoto et al., 1980). We noted that the synthetic methyl derivatives of distearin were practically inactive, but its butyl or phenyl derivatives became PKC activators that were as potent as diolein (Q. Zhou, M. G. Wood, R. L. Raynor, F. Menger, and J. F. Kuo, unpublished results). These findings illustrated further the importance of branched-chain fatty acid moieties in imparting to certain lipids unique physicochemical properties critical for the regulation of PKC. Bazzi and Nelsestuen (1987a,b) reported recently that interactions of substrate proteins with PS and/or Ca^{2+} are essential for their phosphorylation by PKC. It is of interest, therefore, to examine whether the inhibitory and stimulatory effects of various DSPC analogues would be qualitatively and quantitatively related to their abilities to modify such interactions.

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Influence of Head-Group Interactions on the Miscibility of Synthetic, Stereochemically Pure Glycolipids and Phospholipids†

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ABSTRACT: Phase diagrams of binary mixtures of the glycoglycerolipids 1,2-di-*O*-tetradecyl-3-*O*-β-D-galactosyl-*sn*-glycerol (14-Gal) and 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucosyl-*sn*-glycerol (14-Glc) with the phospholipids L-dimyristoylphosphatidylcholine (DMPC) and L-dimyristoylphosphatidylethanolamine (DMPE) were recorded by high-sensitivity differential scanning calorimetry and used for determination of the glycolipid–phospholipid miscibility in solid and liquid-crystalline states. As a consequence of a metastable behavior of both glycolipids and DMPE, the solid-state glycolipid/phospholipid miscibility was strongly dependent on the temperature prehistory of the samples. While DMPC and 14-Glc mix continuously, the other three binaries display extended regions of solid–solid-phase separation in the equilibrium low-temperature states. The DMPE/glycolipid phase diagrams were of clearly expressed eutectic type. Continuous solutions were formed in the liquid-crystalline and in the metastable solid phases of the mixtures. Simulations of the shape of the phase diagrams using the Bragg–Williams approximation showed certain deviations from ideal mixing in the liquid-crystalline continuous solutions. Since both glycolipids and phospholipids contain fully saturated fatty acids of equal chain length, their mixing properties were predominantly determined by the interactions between the lipid polar moieties, assuming the influence of ester or ether linkages of the alkyl chains on the mixing parameters to be negligible. The clearly expressed differences in the mixing of 14-Glc and 14-Gal with phospholipids are most probably due to different hydrogen-bond networks formed by the glucosyl and galactosyl residues.

The glycoglycerolipids constitute one of the major glycolipid classes. They are found in the membranes of plant cells (Sastry, 1974; Quinn & Williams, 1978), animal cells (Sweeley et al., 1977), and many kinds of bacteria (Ward, 1981; Boggs, 1980). Mostly present as minor lipid components, their amount in some membranes can reach remarkably high levels. Galactolipids, for example, represent up to 75% of the total lipid content in chloroplast membranes (Nishihara, 1980). Various functions have been proposed for these lipids, among them maintenance of membrane fluidity, modulation of protein conformations, and external membrane surface receptors (Ishizuka et al., 1985).

Much of the recent progress in the characterization of the structures formed by these amphiphiles in water has been made possible by development of methods for synthesis of stereochemically pure glycoglycerolipids of uniform fatty acid composition (Endo et al., 1982; Six et al., 1983). Application of NMR[1] (Jarrel et al., 1986), X-ray diffraction (Mannock et al., 1985), and DSC (Hinz et al., 1985) on pure glycoglycerolipid/water systems has revealed important aspects of their phase behavior and membrane-forming ability. Nevertheless, in comparison to phosphoglycerolipids, the phase properties of the glycoglycerolipids are less well understood although these lipids have identical lipophilic moieties. A main reason for this lack of understanding is the extreme diversity of the glycolipid carbohydrate composition, which results in a large variety of head groups of different size and polarity. The diversity of the carbohydrate moieties suggests an important contribution of the head-group interactions not only to the bilayer-forming ability of the various glycoglycerolipids but also to their interactions with other lipids and to their vectorial intramembrane localization.

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[1] Abbreviations: 14-Gal, 1,2-di-*O*-tetradecyl-3-*O*-β-D-galactosyl-*sn*-glycerol; 14-Glc, 1,2-di-*O*-tetradecyl-3-*O*-β-D-glucosyl-*sn*-glycerol; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; L-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DL-DPPE, *rac*-1,2-dipalmitoylglycero-3-phosphoethanolamine; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance.

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