Biochimica et Biophysica Acta, 1112 (1992) 251–258

BBAMEM 75810

Interaction of synthetic glycophospholipids with phospholipid bilayer membranes

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(Received 24 April 1992)
(Revised manuscript received 17 August 1992)

Key words: Liposome; Glycophospholipid; Phase transition; Membrane fluidity

A series of glycophospholipids synthesized by coupling mono-, di-, or tri-saccharides to dioleoylphosphatidylethanolamine (DOPE) by reductive amination was used to investigate the interaction of glycophospholipids with phospholipid bilayer membranes. These synthetic glycophospholipids functioned as a stabilizer for the formation of DOPE bilayer vesicles. The minimal mol% of glycophospholipid needed to stabilize the DOPE vesicles were as follows: 8% *N*-neuraminlactosyl-DOPE (NANL-DOPE), 20% *N*-maltotriosyl-DOPE (MAT-DOPE), 30% *N*-lactosyl-DOPE (Lac-DOPE), and 42% *N*-galactosyl-DOPE (Gal-DOPE). The estimated hydration number of glycophospholipid in reverse micelles was 87, 73, 46, and 14 for NANL-DOPE, MAT-DOPE, Lac-DOPE, and Gal-DOPE, respectively. Thus, the hydration intensity of the glycophospholipid was directly related to the ability to stabilize the DOPE bilayer phase for vesicle formation. Glycophospholipids also reduced the transition temperature from gel to liquid-crystalline phase (T_m) of dipalmitoylphosphatidylcholine (DPPC) bilayers. Interestingly, incorporation of NANL-DOPE induced a decrease of membrane fluidity of DPPC bilayers in the gel phase while other glycophospholipids had no effect. Also, low level of NANL-DOPE but not other glycophospholipids increased the transition temperature (T_H) from liquid-crystalline to hexagonal phase of dielaidoylphosphatidylethanolamine bilayers. These results showed that NANL-DOPE with a highly hydratable headgroup which provides a strong stabilization activity for the L_α phase of phospholipid membranes, may also be involved in specific interactions with neighboring phospholipids via its saccharide moiety.

Introduction

Glycolipids are involved in many biological processes including cellular recognition, cell surface receptors for toxins,drugs, natural agonists, and lectins, as well as immune events and others (for reviews, see Refs. 1–4). These functions are largely based on the saccharide structure of the glycolipids, but the ceramide moiety of the molecule also provides an unusual physical property for the cell membranes. The antigenesity of glycosphingolipids is dependent not only on the spatial configuration of the saccharide unit but on the ceramide portion of the lipid as well [5]. Also, it has been shown that glycolipids cluster to form rigid domains in bilayer membranes, possibly due to their extensive H-bonding capability [6] as well as the interdigitated hydrocarbon chains of the ceramide molecules [7]. These rigid domains affect a number of important physical properties of the glycolipids including the conformation of the saccharide moiety [8], and the exit rate of the glycolipid in an exchange reaction [9,10]. The potential biological significance of these properties has been proposed [11]. The functional role of glycolipids as a structural element is also determined by the nature of the sugar headgroup [6].

In a structure-function study on the cryoprotection activity of glycolipids on phospholipid membranes, we have synthesized a series of phosphatidylethanolamine derivatives by coupling mono-, di-, or tri-saccharides via reductive amination [12]. The cryoprotective activity was correlated to the size of the saccharide moiety of

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Abbreviations: DEPE, dielaidoylphosphatidylethanolamine; DOPE, dioleoylphosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; Gal-DOPE, *N*-galactosyl-DOPE, Galβ1 → DOPE; Lac-DOPE, *N*-lactosyl-DOPE, Galβ1 → 4Glcβ1 → DOPE; MAT-DOPE, *N*-maltotriosyl-DOPE, Glcβ1 → 4Glcβ1 → 4Glcβ1 → DOPE; NANL-DOPE, *N*-neuraminylactosyl-DOPE, NeuAcα2 → 4Galβ1 → 4Glcβ1 → DOPE; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

the glycophospholipid with trisaccharide-PE conjugates exhibiting the highest activity. Interestingly, the monosacchride-PE conjugate not only lacks the cryoprotection activity, but also antagonizes the activity of trehalose, a well-established cryoprotectant. Fourier transform infrared studies revealed that trehalose did not H-bond with the phosphate group of the monosaccharide-PE conjugates, which could be the basis of the antagonistic effect.

In the present study, the interactions of these synthetic glycophospholipid conjugates with phospholipid membranes have been systematically investigated. Stabilization of the bilayer structure of unsaturated PE by incorporation of glycophospholipid is demonstrated by vesicle formation and by the entrapment of a water-soluble dye in the vesicles. Perturbation of transition from the gel to the liquid-crystalline phase of DPPC bilayers by the glycophospholipids has also been studied by differential scanning calorimetry and fluorescence depolarization of diphenylhexatriene (DPH). The change of the hexagonal phase transition temperature of DEPE bilayers by the glycophospholipid has been examined by differential scanning calorimetry. The results of these studies should provide some insight to the interactions of glycolipids with phospholipid membranes.

Materials and Methods

Materials. DOPE, DEPE, and DPPC were purchased from Avanti Polar Lipids Inc. (Birmingham, AL.). Calcein, galactose, lactose, maltotriose, and *N*-acetylneuraminylactose were purchased from Sigma. Diphenylhexatriene (DPH) was purchased from Aldrich. [^{3}H]H_2O was purchased from Amersham International (Arlington Height, IL).

Synthesis of saccharide-DOPE conjugates. A series of saccharide-DOPE conjugates was synthesized by the reductive amination coupling method previously reported [12]. Briefly, each saccharide (galactose, lactose, maltotriose, or *N*-acetylneuraminylactose) was dissolved in 20 mM phosphate buffer (pH 7.5). The saccharide solution was mixed with DOPE dissolved in methanol. The molar ratio of sugar to lipid was 50:1 and the volume ratio of phosphate buffer to methanol was 1:10. An excess amount of $NaCNBH_3$ was added to the reaction mixture every 8 h during 48 h incubation at 40°C with constant agitation. The reaction mixture was shaken with a mixture of water and chloroform. The saccharide-DOPE conjugates in the organic phase were harvested and further purified using preparative thin-layer chromatography on silica plates with the solvent system of chloroform/methanol/water (60:35:5, v/v). Purified conjugates run as single spots on high performance thin layer chromatography using the same solvent system. Purified conjugates were quantitated by the phosphate assay [13] and sugar assays [14]. The ratios of phosphate to saccharide in all conjugates were approx. 1. All conjugates were also characterized by FTIR confirming the appearance of -OH stretching and the disappearance of -NH_2 stretching.

Vesicle preparation by sonication. Mixtures of varying amounts of glycophospholipid and DOPE were dried under N_2 stream and vacuum-desiccated for 30 min. PBS (pH 7.5) containing 50 mM calcein was added to the lipid mixture. The lipid suspension (5 mM in total lipid) was sonicated at room temperature using a bath-type sonicator (Laboratory Supplies, Hicksville, NY) to produce unilamellar vesicles. After 1 h incubation at room temperature, half of the vesicle preparation was utilized for 90° light scattering measurement at 660 nm to show vesicle formation using a Perkin-Elmer LS5 spectrofluorometer. The rest was applied to a Bio-Gel A 0.5m column which was eluted with an isotonic PBS in order to remove the free unentrapped calcein. The recovered vesicles were then used for the fluorescence measurement to determine calcein entrapment. The fluorescence intensity of each sample was measured with the same spectrofluorometer ($\lambda_{ex} = 490$ nm, $\lambda_{em} = 520$ nm). The total fluorescence intensity of each sample was obtained by the addition of 50 μl deoxycholate (5%) to lyse the vesicles. Percent fluorescence quenching of calcein entrapped in vesicles was calculated as:

$$\% \text{ quenching} = \frac{F_t - F_o}{F_t} \times 100$$

where F_t is the total fluorescence intensity and F_o is the observed fluorescence intensity of the vesicles.

Vesicle preparation by detergent-dialysis. Lipid samples containing egg PC and glycophospholipids (3:1, molar ratio) were dried under N_2 stream and vacuum-desiccated for 30 min. 200 mM octyl glucoside in PBS (pH 7.5) was added to the lipid suspension (10 mM) which was sonicated briefly until clarity and then dialyzed against PBS (pH 7.5). Vesicle size was estimated by quasi-elastic light scattering using a Coulter N4SD submicron particle analyzer (Coulter, Hialeah, FL) and by negative-stain electron microscopy using 1% uranium acetate as a stain [15].

Fluorescence anisotropy of diphenylhexatriene (DPH). Lipid samples containing DPPC and a various amount of glycophospholipid were dried under N_2 stream and vacuum-desiccated for 30 min. All lipids (2 mmol) were hydrated with 2 ml PBS (pH 7.5) at 45°C for 10 min. 2 ml of 2 mM DPH solution in tetrahydrofuran were added to the sample which was additionally incubated for 3 h at 45°C and then cooled to room temperature. The fluorescence of DPH in each lipid suspension was measured using a Perkin-Elmer LS5 spectrofluorom-

eter ($\lambda_{ex} = 357$ nm, $\lambda_{em} = 430$ nm) equipped with a pair of plane polarizers. Fluorescence polarization was calculated using the following equation:

$$p = \frac{VV - \frac{VH \times HV}{HH}}{VV + \frac{VH \times HV}{HH}}$$

where VV, VH, HV, and HH are the fluorescence intensities measured at four different combinations of the polarizer position (V = vertical, H = horizontal). The fluorescence anisotropy was calculated as $r = 2p/(3-p)$.

Differential scanning calorimetry. DPPC or DEPE samples containing various mol% of glycophospholipid were dried under N_2 stream and vacuum-desiccated for 30 min. 30 μmol lipids were hydrated in 3 ml PBS (pH 7.5) at 45°C for 4 h and then left overnight at room temperature for equilibration. Heating scans of the lipid samples were performed from 25°C to 90°C with a scan rate of 20 C°/h using a MC2 high-sensitivity differential scanning calorimeter (Microcal, Amherst, MA). T_m (transition temperature, L_β to L_α), T_H (transition temperature L_α to H_{II}) and enthalpy change were determined by a computer interfaced with the calorimeter.

Measurement of headgroup hydration in reverse micelles. A method for direct measurement of headgroup hydration of polar lipid in reverse micelles was developed by Sen and Hui [16]. Briefly, 1 mg each glycophospholipid was dried under N_2 stream and vacuum-desiccated for 2 h. 1 ml pure dry hexane and 25 μl ^{3}H-labeled water (25 μCi/ml) were added to the dried lipid. The two phases were mixed vigorously using a vortex mixer and then sonicated for 20 min in a bath type sonicator. After two phases were separated, an aliquot of the hexane phase was used for determination of radioactivity with a liquid scintillation counter. Another aliquot of the same hexane phase was used for phosphate assay [13]. The number of water molecules associated per lipid molecule was calculated from the data.

Results

Stabilization of DOPE bilayers by glycophospholipid

Formation of stable DOPE bilayer vesicles by incorporating synthetic glycophospholipid was monitored by two different methods: 90° light scattering and fluorescence quenching of calcein entrapped in the vesicles. Formation of stable vesicles results in a low level of light scattering [17]. Also, stable vesicles entrapping 50 mM calcein normally exhibit approx. 70% fluorescence quenching [18]. In order to determine the minimal mol% of synthetic glycophospholipid needed to stabilize the DOPE bilayer vesicles, various amounts of the glycophospholipid were incorporated into the lipid composition (Fig. 1). The estimated minimal mol% of each glycophospholipid determined by the two methods was quite similar: 8% NANL-DOPE, 20% MAT-DOPE, 30% Lac-DOPE, and 42% Gal-DOPE. This pattern of the minimal mol% directly represented the order of stabilization activity of the glycophospholipid for the formation of the DOPE bilayer vesicles. Glycophospholipids with a larger saccharide moiety showed better stabilization activities. Among the glycophospholipids, the monosaccharide-DOPE (Gal-DOPE) had the poorest activity and the disaccharide-DOPE (Lac-DOPE) was the next. Moreover, NANL-DOPE was a better stabilizer than MAT-DOPE although both had trisaccharide headgroups. This may be due to the additional negative charge of the terminal sialic acid on the headgroup of NANL-DOPE.

Hydration number and vesicle curvature

Hydration intensity around the lipid headgroup and the hydration force in the interfacial region are major factors affecting lipid polymorphism [19]. Recently, a method for direct measurement of headgroup hydration of phospholipid in reverse micelles has been developed [16]. Hydration number of phospholipid measured by this method were in close agreement with those obtained from other measurements using phos-

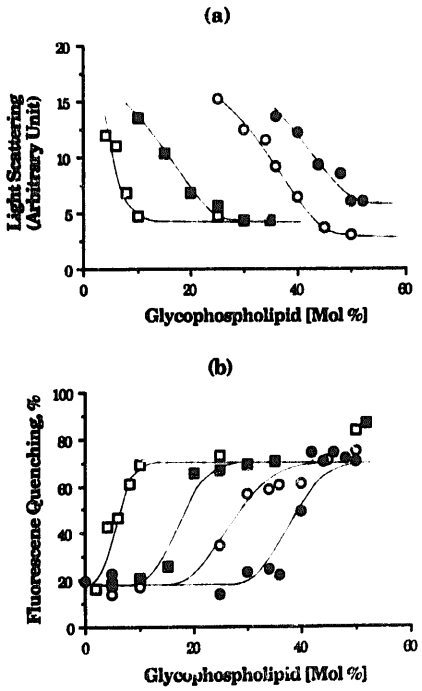

Fig. 1. DOPE bilayers stabilization by synthetic glycophospholipids. (a) 90° light scattering of the sonicated liposomes containing various amount of Gal-DOPE (●), Lac-DOPE (○), MAT-DOPE (■), or NANL-DOPE (□). (b) Percent fluorescence quenching of calcein entrapped in the same liposomes.

TABLE I

Hydration number and effect of glycophospholipid on liposome size

Lipid	Hydration number [a]	Liposome diameter (nm) [b]	
		QELS [c]	EM [d]
None	–	196 (47)	210 (65)
Cholesterol	2.5 (0.3)	292 (60)	310 (45)
Gal-DOPE	14.4 (2.5)	164 (38)	196 (35)
Lac-DOPE	46.5 (6.7)	138 (46)	159 (21)
MAT-DOPE	73.1 (8.8)	124 (38)	141 (60)
NANL-DOPE	87.0 (9.1)	75 (22)	46 (8)

[a] Hydration number of glycophospholipid was measured in the reverse micelle phase. Mean (S.D.), $n = 3$.

[b] Liposomes composed of egg PC/glycophospholipid or cholesterol (3:1, molar ratio) prepared by a dialysis method (detailed in Methods and Materials).

[c] Measured with a Coulter N4SD submicron particle analyzer.

[d] Measured by electron microscopy using 1% uranium acetate as negative stain. At least 100 vesicles were measured in each preparation. Mean (S.D.).

pholipids in the lamellar phase. As expected, hydration number of the synthetic glycophospholipid increased with the size of their sugar moieties (Table I). For trisaccharide-DOPE's, the hydration number of NANL-DOPE was larger than that of MAT-DOPE, which must be due to the negative charge of the terminal sialic acid in NANL-DOPE. The hydration number of Lac-DOPE was between those of MAT-DOPE and Gal-DOPE. As a control measurement, the hydration numbers of egg PC and DOPE were determined by the same method and found to be 25.3 and 9.3, respectively. These numbers are in close agreement with those previously reported by others [20,21].

One of the major factors determining the vesicle curvature is the hydration force in the interfacial area of membrane [19]. The hydration number measured above was clearly related to the vesicular curvature of the lipid bilayers containing glycophospholipid (Table I). Vesicles prepared by the detergent dialysis method are at a relatively low energy state because minimal mechanical force is involved during the process. The size difference between vesicles prepared under the same conditions should reflect the difference in the intrinsic property of glycophospholipid affecting the vesicle curvature. Vesicle size was measured by two different methods: quasi-elastic light scattering (QELS) and electron microscopy using negative staining (micrographs not shown). The size measurement of egg PC vesicles containing 25 mol% of glycophospholipid showed an inverse relationship to the hydration number of the glycophospholipid. Thus vesicles containing NANL-DOPE, the most hydratable glycophospholipid, had smallest mean diameter, whereas vesicles containing Gal-DOPE, the least hydratable glycophospholipid, had the largest mean diameter.

Effect of glycophospholipid on main phase transition and membrane fluidity of DPPC

Depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) is commonly used as a means of estimating membrane fluidity as well as revealing the phase transition from a gel to a liquid-crystalline state [22,23]. Fluorescence anisotropy of DPH is sensitive to the structural order of lipids in the membrane. The temperature dependence of fluorescence anisotropy of DPH was studied with DPPC bilayer vesicles containing various amount of glycophospholipid (Fig. 2). Generally, addition of glycophospholipid resulted in a decrease in T_m and a broadening of the transition of DPPC bilayers. Decrease in T_m by the addition of Gal-DOPE was larger than what was observed for NANL-DOPE at low mol% (Fig. 2a). However, the addition of 40% NANL-DOPE seemed to abolish the clear phase transition or decreased it to below 25°C, whereas an equal amount of Gal-DOPE reduced the T_m to about 30°C (Fig. 2b). The T_m change of DPPC bilayers by Lac-DOPE or MAT-DOPE was similar to what was observed for Gal-DOPE (data not shown).

Interestingly, addition of 5 mol% NANL-DOPE induced a reproducible increase of DPH anisotropy in the gel state of DPPC, whereas Gal-DOPE did not affect DPH anisotropy in the same state (Fig. 2a). At 40 mol%, both glycophospholipids induced a reduction of the DPH anisotropy (Fig. 2b). These results indicate that a relatively low mol% of NANL-DOPE, but not Gal-DOPE, has an ordering effect on DPPC bilayers in

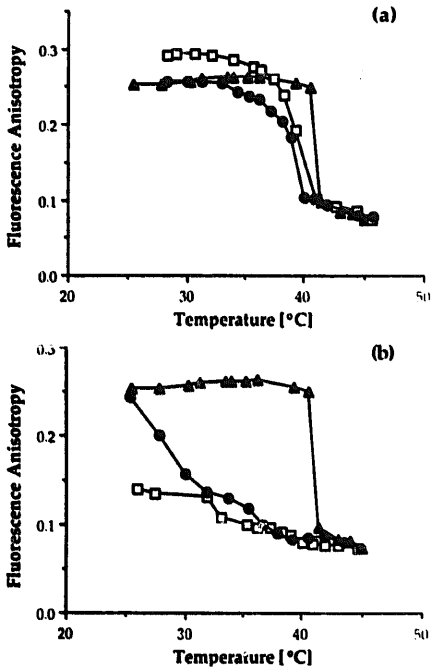

Fig. 2. Fluorescence anisotropy of DPH in DPPC bilayers containing various glycophospholipids. Fluorescence anisotropy of DPH in DPPC bilayers containing none (▲), Gal-DOPE (●), or NANL-DOPE (□) was measured at various temperature. Graph (a), 5 mol% glycophospholipids. Graph (b), 40 mol% glycophospholipids.

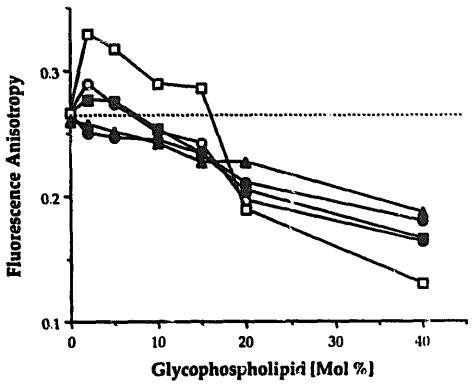

Fig. 3. Fluorescence anisotropy of DPH in DPPC bilayers containing various glycophospholipids in the gel phase. Fluorescence anisotropy of DPH in DPPC bilayers containing Gal-DOPE (●), Lac-DOPE (○), MAT-DOPE (■), NANL-DOPE (□) or DOPE (▲) was measured at 25°C. The horizontal dotted line represents the anisotropy for the pure DPPC bilayers.

the gel phase. Lac-DOPE and MAT-DOPE showed almost the same effects as that of Gal-DOPE (data not shown). Addition of glycophospholipids did not affect the DPH fluorescence anisotropy of DPH in DPPC bilayers in the liquid-crystalline state.

In order to examine such an unusual action of NANL-DOPE in DPPC bilayers in the gel phase, the same procedure was repeated with varying amounts of synthetic glycophospholipids at 25°C where the DPPC bilayers exist in the gel state (Fig. 3). Addition of NANL-DOPE up to 15% induced the fluidity decrease in DPPC bilayers. Lac-DOPE and MAT-DOPE induced a small decrease below 5 mol%. Steady decrease in anisotropy was observed for Gal-DOPE and DOPE at all molar ratios tested. These data suggest that the saccharide headgroups of glycophospholipids affect the acyl chain order of the DPPC bilayer membrane, which is sensed by the probe DPH. The fluidity increase of DPPC bilayers by 40 mol% NANL-DOPE was greater than what was observed for any other glycophospholipids at the same mol%.

DSC studies of the effect of glycophospholipids on main phase transition of DPPC

Differential scanning calorimetry (DSC) is a sensitive method to monitor changes of phase transition parameters (ΔT_m, ΔT_H, ΔH, and ΔS). Effects of NANL-DOPE and Gal-DOPE on the T_m of DPPC bilayers were compared to each other at the same mol% (Fig. 4). Curve (a) in Fig. 4 is a typical scan of pure DPPC bilayers, which shows a clear phase transition from gel (L_β) to liquid-crystalline (L_α) at 42°C and a pretransition at 36°C. Incorporation of Gal-DOPE or NANL-DOPE into DPPC bilayers reduced the T_m of DPPC and abolished the pretransition. The ΔT_m induced by Gal-DOPE was greater than that by NANL-DOPE. These data are comparable with those of Fig. 2. The peak of phase transition became broader with an increase of the glycophospholipid content. ΔH and ΔS were also calculated from the DSC data. Table II contains data for T_m, ΔH, and ΔS at each lipid composition. DPPC/NANL-DOPE bilayers showed larger ΔH and ΔS during the phase transition than the pure DPPC bilayers. However, Gal-DOPE appeared not to

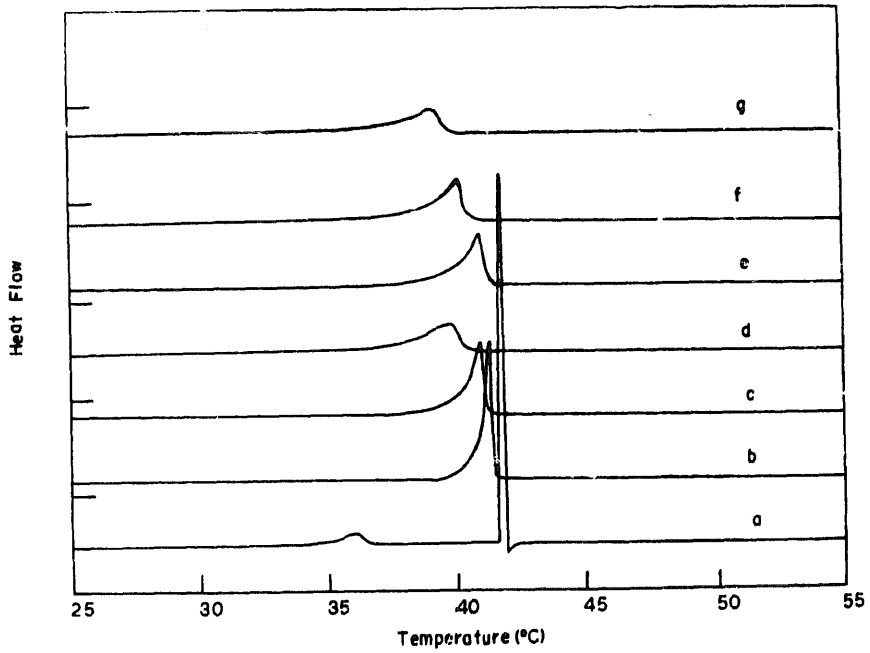

Fig. 4. Effect of glycophospholipid on the DSC scan of DPPC bilayers. Scans of DPPC bilayer samples containing (a) none, (b) 2% NANL-DOPE, (c) 4% NANL-DOPE, (d) 6% NANL-DOPE, (e) 2% Gal-DOPE, (f) 4% Gal-DOPE, or (g) 6% Gal-DOPE are shown.

TABLE II

Effect of glycophospholipid on the phase transition of DPPC bilayers

Glycophospholipid [a]		T_m (°C)	ΔH(kcal/mol)	ΔS(cal/mol per deg)
None		41.7	8.7	27.8
Gal-DOPE	2%	40.9	9.1	29.0
	4%	40.1	8.8	28.1
	6%	39.1	7.9	24.3
NANL-DOPE	2%	41.3	10.1	32.3
	4%	40.9	10.4	33.1
	6%	39.7	9.9	31.5

[a] DPPC containing various amount of glycophospholipid was used for differential scanning calorimetry measurements as described in Materials and Methods.

affect ΔH and ΔS of the DPPC bilayers although it effectively lowered the T_m.

Effect of glycophospholipid on hexagonal phase transition of DEPE

The DSC analysis was also used to study the effect of glycophospholipid on the phase transition from L_α to H_{II} of DEPE (Fig. 5). It has been shown that a L_α phase-stabilizing amphiphilic molecule increases the hexagonal phase transition temperature (T_{II}) of DEPE, whereas a destabilizing one decreases the T_{II} [24,25]. Curve (a) in Fig. 5 is a typical thermal scan of DEPE, showing a main transition ($L_\beta \rightarrow L_\alpha$) at 37°C and a hexagonal phase transition ($L_\alpha \rightarrow H_{II}$) at 66°C. Addition of 4 mol% NANL-DOPE induced an increase of the T_H of DEPE to 74°C and a decrease of the T_m down to 36.5°C. These data indicate that NANL-DOPE functions as an amphiphilic stabilizer for the L_α phase of DEPE bilayers. However, addition of 4% Gal-DOPE lowered the T_m to 35.5°C and also lowered the T_H to 63°C. This indicates that Gal-DOPE functions as a H_{II} phase stabilizer. The T_H change of DEPE upon adding various mol% of glycophospholipid is shown in Fig. 5(inset). The T_H of DEPE increased with the mol% of NANL-DOPE, but decreased at all mol% of Gal-DOPE tested. Even though Gal-DOPE was a stabilizer to form DOPE bilayers (Fig. 1), it did not function as a stabilizer for the L_α phase of DEPE bilayers. The hexagonal phase transition of DEPE bilayers containing more than 6 mol% of Gal-DOPE was too broad to be recognized. Changes in the phase transition of DEPE bilayers by addition of Lac-DOPE or MAT-DOPE were quite similar to those observed with Gal-DOPE (data not shown).

Discussion

Glycolipids are structurally diverse and are major components of the cell membrane. While most studies on glycolipids are aimed at elucidating their functions as cell surface receptors, the physical properties of glycolipid in phospholipid membranes are not well established. In the present study we have systematically investigated the interaction of synthetic glycophospholipids with phospholipid bilayer membranes. For this

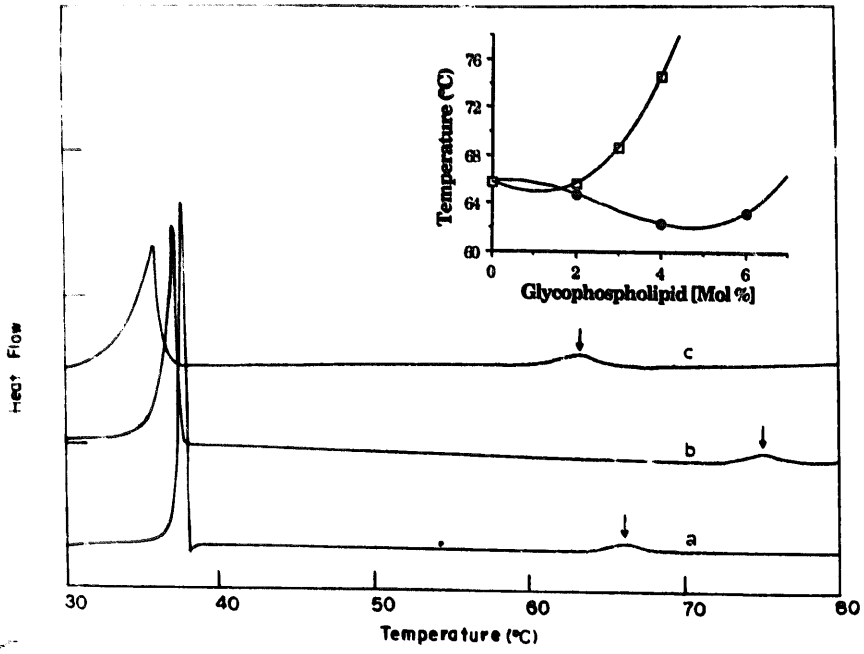

Fig. 5. Effect of glycophospholipid on DSC scan of DEPE bilayers. Scan of DEPE bilayers containing (a) none, (b) 4% NANL-DOPE, or (c) 4% Gal-DOPE are shown. The peak temperature of the hexagonal phase transition is marked with arrow. The inset shows the effect of glycophospholipid on the T_H of DEPE bilayers containing various amount of Gal-DOPE (●) or NANL-DOPE (□) measured by DSC.

investigation, mono-, di-, or tri-saccharides have been conjugated to DOPE, a common lipid anchor, by reductive amination. The glycophospholipids would be expected to resemble the corresponding glycolipids with one or two additional sugar residues in terms of hydration capacity, phase transition temperature, and the ability to form nonlamellar mesophases. Therefore, the examination of synthetic glycophospholipid–phospholipid interactions should lend support to the previous studies using natural glycolipids [26,27].

These synthetic glycophospholipids differ from each other mainly in terms of the headgroup hydration (Table I). The degree of hydration was directly proportional to the number of the neutral saccharide unit of the headgroup, with an increase of hydration number of approx. 30 H_2O molecules for each additional neutral saccharide unit. The presence of sialic acid in NANL-DOPE gave additional hydration numbers due to the negatively charged carboxyl group.

Due to the ability to increase the headgroup hydration, these glycophospholipids showed various activities to stabilize the DOPE bilayer vesicles. The activity was directly related to the hydration number of the glycophospholipid (Fig. 1). Increased interfacial hydration would increase the repulsive force when vesicles are brought to close contact during collision. The repulsive force is a major kinetic barrier for the destabilization of DOPE vesicles in aqueous suspension [28–30]. Interfacial hydration is also a major factor in determining the curvature of bilayers at equilibrium [31]. Highly hydrated bilayers may curve to a greater extent, hence forming smaller vesicles, than the poorly hydrated bilayers (Table I).

We have chosen NANL-DOPE and Gal-DOPE for further detailed studies because they represent the extreme cases of hydration intensity of the glycophospholipids. Incorporation of both glycophospholipids into DPPC bilayers has brought the expected effect of decreasing the cooperativity of the $L_\beta \rightarrow L_\alpha$ phase transition which was shown by both DPH fluorescence anisotropy (Fig. 2) and DSC (Fig. 4 and Table II). Effects of broadening of the phase transition and decrease of the T_m were more pronounced with Gal-DOPE than NANL-DOPE (Figs. 2–4 and Table II). The strong hydration intensity of NANL-DOPE may have partially stabilized the L_α phase of DPPC bilayers which is known to be more hydrated than the L_β phase [32]. Addition of Gal-DOPE to DPPC bilayers should decrease the average hydration level of the bilayers as this glycophospholipid only binds with 14 H_2O molecules which are approximately half of the number for the PC molecule [16,20]. These data have demonstrated the important role of interfacial hydration on the $L_\beta \rightarrow L_\alpha$ phase transition.

Similar effect of hydration could also be observed on the influence of $L_\alpha \rightarrow H_{II}$ phase transition of DEPE by the two glycophospholipids. This phase transition is highly sensitive to the incorporation of exogenous amphiphiles [24,25]. Gal-DOPE, with which the hydration intensity is not much different from that of the PE itself [16,21], destabilized the L_α phase by lowering the T_H (Fig. 5). On the other hand, the highly hydrated NANL-DOPE has brought a stabilization effect on the L_α phase by elevating the T_H (Fig. 5). Similar stabilization effect has been reported for amphiphiles possessing a charged headgroup which significantly increase the hydration intensity of the molecule [24,25].

Somewhat unexpected was the effect of NANL-DOPE on the gel (L_β) phase of DPPC (Figs. 2 and 3). A reproducible increase in the DPH fluorescence anisotropy was observed at temperatures below T_m for DPPC bilayers containing various amounts of NANL-DOPE, indicating that the lipids in the gel phase are more ordered due to the presence of this glycophospholipid. This is not likely an artifact of using DPH as a probe, because the transition enthalpy (ΔH), and entropy (ΔS) were also increased by about 15–18% at low level NANL-DOPE incorporation as measured by DSC (Table II). This phenomenon could be related to an earlier observation made by Tsao et al. [33] who reported that ganglioside G_{D1a} at low mol% could enhance the ordering of PE bilayers. Since both NANL-DOPE and G_{D1a} contain sialic acid residues in their headgroups, specific interactions with the neighboring headgroups of phospholipids may bring about an enhanced interlipid interaction. ^{2}H-NMR and crystal structure studies have revealed strong intermolecular H-bondings which restricted the motion of the glycosyl headgroups of glycolipid in gel state membranes [34,35]. Physical properties of glycolipid such as the acyl chain order and phase transition temperature, can be attributed to the H-bonding network of the headgroups [36,37]. The fluidity of the apolar interior of a bilayer decreases with increasing ganglioside content [36] and with a cooperative glycosyl headgroup interaction [37]. The motion of a glycolipid headgroup is much slower than that of a phospholipid headgroup and the reduced mobility of the saccharide group may thus affect the mobility of the acyl chain [38–41]. Fenske et al. [42] have recently suggested that sialylglycerolipids may alter the headgroup orientation of phospholipid and increase the rate of orientational exchange in mixed model membranes due to an increase in the rate of lateral diffusion of the phospholipid.

In summary, this study has demonstrated the role of saccharide headgroups of glycolipids in phospholipid polymorphism. We believe that most of the observed effects can be attributed to the hydration intensity of the saccharide headgroup. However, the negatively charged sialic acid residue of a glycolipid may influence the physical properties of phospholipid by more

than just the high level of headgroup hydration. Specific interactions such as H-bondings with the neighboring phospholipids should also be taken into consideration. This hypothesis will be tested in future studies.

Acknowledgement

This work was supported by NIH grant CA 24553.

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