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Modulation by gangliosides of the lamellar-inverted micelle (hexagonal II) phase transition in mixtures containing phosphatidylethanolamine and dioleoylglycerol

(calorimetry/^{31}P-NMR/pyrene fluorescence)

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ABSTRACT **We studied the effect of gangliosides GD1a and GM1 on the lamellar-to-hexagonal II phase transition of mixtures of dioleoylphosphatidylethanolamine/dioleoylphosphatidyl choline, 3:1, and of transphosphatidylated phosphatidylethanolamine with dioleoylglycerol by high-sensitivity differential scanning calorimetry, ^{31}P-NMR, and pyrene fluorescence of a phosphatidylcholine probe. Gangliosides had a dual effect. Below 1 mol % ganglioside the hexagonal II phase transition was affected but still occurred at lower temperature than in the absence of gangliosides. The presence of between 1 and 2 mol % gangliosides increased the temperature for formation of the hexagonal II phase and progressively decreased its cooperativity. Above 3 mol % gangliosides totally inhibited the formation of both the temperature-induced and composition-induced hexagonal phase, probably by opposing the geometric distortions necessary for the inverted micellar structures.**

Packing properties of lipids in bilayer and nonbilayer structures of membranes depend on thermodynamic factors coupled to molecular geometry. This is expressed by the optimal lipid molecular area a_o, the hydrocarbon volume v, and the maximum length of hydrocarbon chains l_c (1, 2). The restrictions imposed on the interfacial curvature derived from the molecular shape are represented in the dimensionless critical packing parameter $P = v/(a_o \cdot l_c)$ (1).

The most common lipids in mammalian cell membranes are the two glycerophospholipids phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) of moderate unsaturation. Their critical packing parameter reflects a geometrical shape for PtdCho roughly resembling a truncated cone ($P_{PtdCho} < 1$), whereas PtdEtn packs as an inverted cone ($P_{PtdEtn} > 1$). This is compatible with the packing of PtdCho into bilayers of different curvatures according to the fatty acyl length, unsaturation, and phase state, whereas PtdEtn favors nonbilayer inverted micellar structures [hexagonal II (H_{II}) type] (1, 3). Even simple binary aggregates of these lipids can exhibit rich structural variations with respect to composition and temperature (4, 5).

On the other hand, biological membranes contain variable proportions of many lipid species having different molecular geometries. Their coexistence within a same structure is highly dynamic and is constrained by compositional, thermodynamic, and topologic restrictions (3, 5–7). Structural rearrangements among bilayer and nonbilayer phases participate in many cellular processes mediated by membrane interactions, recombination, and signaling (8, 9). Some lipids are present in small or transient amounts but have large amplified effects on the membrane stability and topology (4, 10). Gangliosides are one group of such lipids normally present below 10 mol % in biomembranes but having important cellular functions (10). We and others have previously described the molecular packing, critical parameters, and thermotropic behavior of gangliosides and their binary mixtures with phospholipids (11–13). Most gangliosides have conical shapes with values of critical packing parameters well out of the range, allowing formation of stable bilayers and, in pure form, these lipids generally form micelles of different shape (11, 14, 15). When mixed with the bilayer-forming PtdCho, gangliosides are incorporated into the bilayer and affect its curvature; if exceeding a certain proportion, the thermodynamic–geometric stress induces reorganization into micelles (7, 11, 14). The effect of gangliosides on the topology of membranes containing H_{II}-forming lipids has not, as far as we know, been previously investigated. In this work we showed by three independent physical methods that ganglioside GD1a and GM1 at low proportions in the lipid mixture markedly affected the formation of the H_{II} phase. Gangliosides had a dual effect, depending on their proportion in the mixture. Their presence at <1 mol % decreased the temperature for the H_{II} phase transition of dioleoylphosphatidylethanolamine [$(Ole)_2$PtdEtn]. Between 1 and 2 mol % gangliosides increased the temperature for formation of the H_{II} phase and progressively decreased its cooperativity. Above 3 mol % gangliosides inhibited the lamellar–H_{II} phase transition of $(Ole)_2$PtdEtn in mixtures with dioleoylphosphatidylcholine [$(Ole)_2$PtdCho] and shifted upward the compositional threshold at which dioleoylglycerol [$(Ole)_2$Gro] induced the H_{II} phase (5, 6, 16, 17) with transphosphatidylated PtdEtn (TPE).

MATERIALS AND METHODS

Gangliosides were purified as described (11, 18); other lipids were from Avanti Polar Lipids [all lipids were >99% pure as judged by high-performance TLC (19)]. After premixed solutions in chloroform/methanol of the lipids in the desired proportions were dried under N_2, the lipid was kept under vacuum for at least 4 hr as reported (7). Lipid dispersions $(Ole)_2$PtdEtn/$(Ole)_2$PtdCho (molar ratio, 3:1) or TPE/$(Ole)_2$Gro [with 0–18 mol % $(Ole)_2$Gro], containing 0, 0.25, 0.5, 1, 2, and 5 mol % gangliosides were prepared daily by mechanical dispersion at room temperature. Cycling steps at low and high temperatures during the dispersion of the lipids in aqueous solutions were omitted. For high-sensitivity dif-

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Abbreviations: PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; $(Ole)_2$PtdCho, dioleoylphosphatidylcholine; $(Ole)_2$PtdEtn, dioleoylphosphatidylethanolamine; $(Ole)_2$Gro, dioleoylglycerol; GM1, Gal(β1-3)GalNac(β1-4)Gal(3-2α)NeuAc(β1-4)Glc(β1-1′)*N*-acylsphingosine; GD1a, NeuAc(α2-3)Gal(β1-3)GalNac(β1-4)Gal(3-2α)NeuAc(β1-4)Glc(β1-1′)*N*-acylsphingosine; B-3782, 1,2-bis[4-(1-pyrene)decanoyl]-*sn*-glycero-3-phosphocholine; HSDSC, high-sensitivity differential scanning calorimetry; TPE, transphosphatidylated PtdEtn (from egg PtdCho); H_{II}, hexagonal II type.

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ferential scanning calorimetry (HSDSC) the lipid dispersions (2–8 mg/ml/10 mM buffer TES [*N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], pH 7.4/10 mM $MgCl_2$/100 mM NaCl/5 mM EDTA) were kept at 0°C for 2 days before scanning with Privalov (Mashpriborintorg, Moscow) DASM-1M or Microcal (Amherst, MA) MC2D calorimeters at a rate of 0.5°C/min, as described (7); similar results were found at a scan rate of 1°C/min. ^{31}P-NMR was done on 10-mm samples containing lipid dispersion at 20–40 mg/ml in ^2H_2O. Spectra were acquired on an IBM AF 270 spectrometer operating at a ^{31}P frequency of 109 MHz with a spectral width of 30 KHz (20). Fluorescence spectra of bispyrenedecanoyl phosphocholine (B-3782; Molecular Probes) (0.1 mol % of total lipid) were recorded in a SLM Aminco (Urbana, IL) model 4800C spectrofluorometer with excitation at 350 nm. The excimer-to-monomer ratio was obtained from the emission at 480 nm and 390 nm (21) of lipid dispersions diluted in 1.5 ml (130 μM total lipid) of the buffer given above for HSDSC. Light scattering was not changed by gangliosides and was always subtracted. All experiments were done at least in triplicate.

RESULTS

Fig. 1*a* shows the ^{31}P-NMR spectrum of a mixture of $(Ole)_2$PtdEtn/$(Ole)_2$PtdCho (molar ratio, 3:1). The spectrum of the phospholipid mixture exhibits the characteristic shift of the signal shoulder from low to high field due to the temperature-induced lamellar-to-H_{II} phase transition. This shift occurs between 40 and 45°C as reported (22). Addition of 0.5 mol % of GD1a (or GM1, data not shown) decreases the temperature at which the transition is detected; the shoulder shift occurs between ≈35 and 45°C (Fig. 1*b*). Above 3 mol % ganglioside the transition is no longer detected, even up to 65°C (Fig. 1*c*).

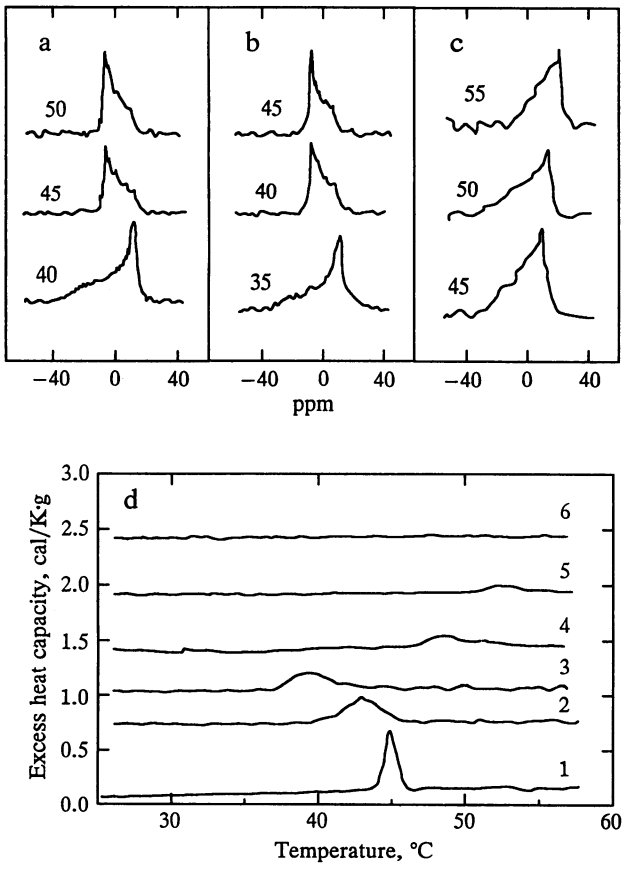

FIG. 1. Effect of ganglioside GD1a on the lamellar-to-H_{II} phase transition. ^{31}P-NMR spectra at the temperatures indicated for mixtures of $(Ole)_2$PtdCho/$(Ole)_2$PtdEtn, 3:1, containing no ganglioside (*a*) or GD1a in the proportion of 0.5 mol % (*b*) and 5 mol % (*c*). (*d*) Calorimetric scans (0.5°C/min) for mixtures of $(Ole)_2$PtdCho/$(Ole)_2$PtdEtn, 3:1, containing no ganglioside (curve 1), or GD1a in the proportion of 0.25 mol % (curve 2), 0.5 mol % (curve 3), 1 mol % (curve 4), 2 mol % (curve 5), and 5 mol % (curve 6).

The HSDSC scan of samples of the same composition as above revealed effects similar to those detected in the ^{31}P-NMR experiments. With <1 mol % gangliosides (Fig. 1*d*, curves 2 and 3) the lamellar-to-hexagonal phase transition occurs ≈3–7°C lower (in different experiments) than that observed in ganglioside-free mixtures (Fig. 1*d*, curve 1). Proportions of 1 and 2 mol % gangliosides progressively broaden and increase the temperature for formation of the H_{II} phase (Fig. 1*d*, curves 4 and 5). When the proportion of gangliosides in the mixture is >3 mol %, the transition becomes undetectable either by ^{31}P-NMR (Fig. 1*c*) or HSDSC (Fig. 1*d*, curve 6). Similar to GD1a the phospholipid lamellar-to-H_{II} transition was also abolished in mixtures of TPE/$(Ole)_2$Gro containing 5 mol % ganglioside GM1 (data not shown).

The H_{II} phase transition of pure $(Ole)_2$PtdEtn has been reported between 8 and 12°C when detected by ^{31}P-NMR or HSDSC (20, 22–24) and is increased to >40°C by inclusion of different proportions of $(Ole)_2$PtdCho (20, 24). Time-resolved depolarization and excimer-formation fluorescence studies with diphenylhexatriene- or pyrene-labeled phospholipids showed that these probes can detect the presence of hexagonal phase in mixtures of different H_{II}-forming lipids (21, 25). With these methods the H_{II} phase transition appears shifted ≈6–10°C higher and extends over a broader range compared with HSDSC. This result has been attributed to a preferential partitioning of the fluorescent probe into the lamellar phase, a geometrical influence of the acyl chain packing on the existence and life time of intramolecular excimers, and the rate of energy exchange and extent between allowed conformational states of the probe in a particular lipid mixture (21). The phase changes detected with the B-3782 probe in our work coincide with those seen by others (21).

Fig. 2 shows the results with bispyrenedecanoyl phosphocholine probe B-3782. Pyrene-excimer formation in a lipid phase depends on collisional diffusion favored by proximity of the pyrenyl groups linked to the fatty acyl chains of the same phospholipid. The very different geometric restrictions of the lamellar compared with the H_{II} phase is reflected in distortions of the lipid packing that affect the rate and extent of pyrene-excimer formation, depending on the lipid composition (21, 25). A relatively large number of points must be collected with this method. The phase transition is shown as an inflection point in the curve describing the variation of the excimer/monomer ratio vs. temperature [or vs. the proportion of $(Ole)_2$Gro for the compositional-induced change] (21). Fig. 2*a* illustrates the probe fluorescence in mixtures with $(Ole)_2$PtdEtn, and Fig. 2*b* shows that of samples containing gangliosides. Similar to what was observed with the other methods, gangliosides >3 mol % inhibit the S-shaped increase of the excimer/monomer ratio occurring as a consequence of the temperature-induced H_{II} phase transition of $(Ole)_2$PtdEtn. Fig. 2*c* shows the isothermal variation of the excimer/monomer ratio in mixtures of TPE containing variable amounts of $(Ole)_2$Gro, a lipid well known to induce formation of the H_{II} phase (5, 16, 22). In the presence of gangliosides (Fig. 2*d*), higher proportions of $(Ole)_2$Gro are necessary for inducing the phase transition in these mixtures. The effects of GM1 were similar to those of GD1a.

DISCUSSION

Complex gangliosides have a large cross-sectional area in relation to the length of the hydrocarbon portion (11, 14, 26).

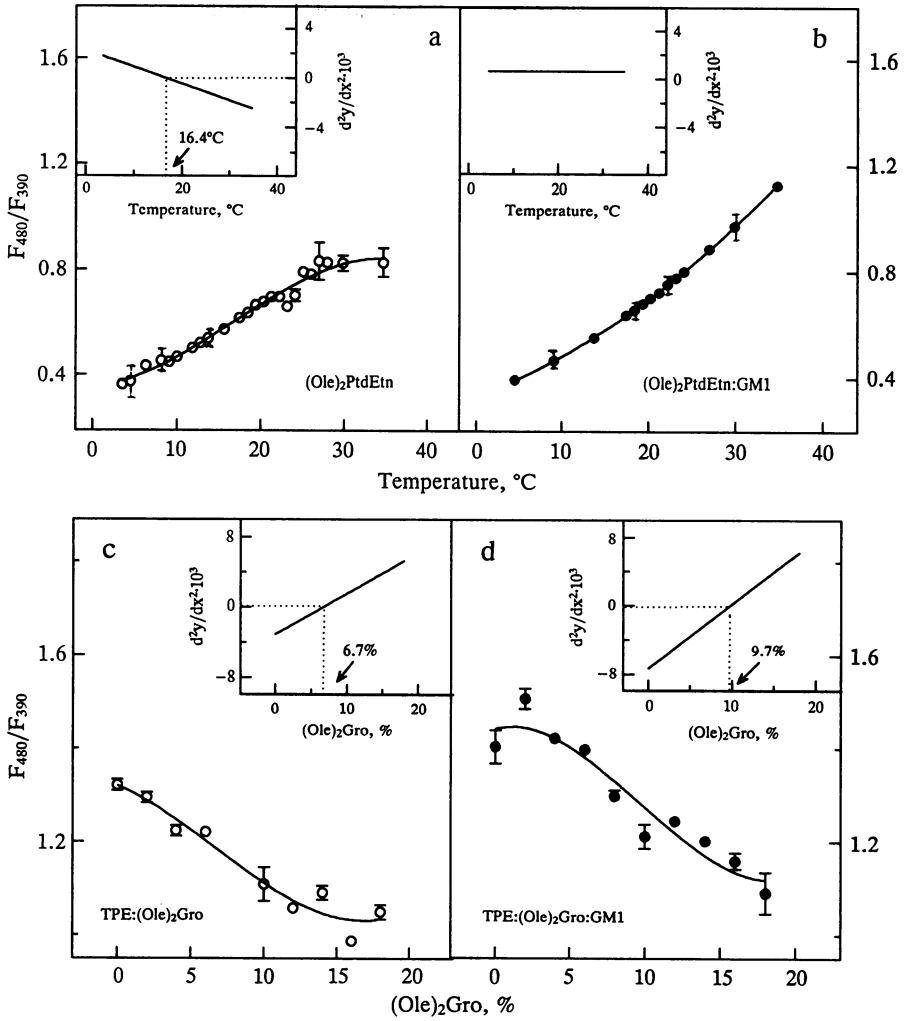

FIG. 2. Effect of ganglioside GM1 on the lamellar-to-H_{II} phase transition. The ratio of the fluorescence (F) at 480 nm (excimer) and 390 nm (monomer) of 0.1 mol % B-3782 probe in a mixture with $(Ole)_2PtdEtn$ in the absence (*a*) or presence (*b*) of 5 mol % GM1 was determined as a function of temperature. The excimer/monomer fluorescence ratio of B-3782 (present at 0.1 mol %) was also measured in a mixture of TPE/$(Ole)_2Gro$, containing (*d*) or not containing (*c*) 5 mol % of GM1, as a function of the percentage of $(Ole)_2Gro$. All points are the mean of 9–12 replicates. The bars (shown only for a few points for clarity) represent the maximum SEM values obtained. To better locate the steepest slope of the curves the experimental points were fitted with the regression line shown (third-order polynomial regression; r^2 was between 0.9683 and 0.998 for all curves); the inflection point was located as the value on the abscissa corresponding to the zero ordinate of the straight line representing the second derivative (*Insets*) of the fitted regression equation.

This conical shape is opposite to the curvature required by inverted micellar H_{II} type of phases (1–6, 22). In the absence of gangliosides ethanolamine-containing phospholipids and $(Ole)_2Gro$ favor the formation of inverted micellar structures (1, 16, 22). As shown in the present work, proportions of gangliosides of 1 and 2 mol % increase and broaden and >3 mol % totally inhibit the formation of the hexagonal phase. The inhibitory effect can be understood in terms of geometrical compensation by gangliosides (11, 14, 15) of the interfacial curvature and topological distortions required by the H_{II} phase. The effect of gangliosides on the H_{II} phase transition may relate to that observed with other conically shaped molecules such as lysolecithin (22). Both the temperature-induced [at constant composition in mixtures with $(Ole)_2PtdEtn/(Ole)_2PtdCho$] and the composition-induced [isothermally, in mixtures with TPE and various $(Ole)_2Gro$] H_{II} phase transition are inhibited by >3 mol % ganglioside. On the other hand, at very low proportions of gangliosides (not >1 mol %) the HSDSC and ^{31}P-NMR experiments reveal that the temperature-induced lamellar-to-H_{II} phase transition still occurs but at lower temperature than in the absence of gangliosides. This effect is usually found for lipids favoring the H_{II} phase (22) and indicates a dual effect of gangliosides, depending on their proportion in the mixture.

Clearly, the dual effect cannot be explained simply on the basis of the individual geometry of the ganglioside molecule. It may be due to the different intermolecular interactions of gangliosides with PtdCho compared with PtdEtn at the local level, in combination with the overall degree of alterations of interfacial curvature that is greatly dependent on the ganglioside proportions (11, 12, 14, 27). Gangliosides show preferential thermodynamic interactions occurring with negative free energy of mixing with the bilayer-forming PtdCho, whereas the free energy of mixing is positive, and thus unfavorable, with the H_{II}-forming PtdEtn (27). On the basis of surface potential measurements it was shown previously that defined dipole–dipole and charge–dipole interactions among the polar-head group of gangliosides and phospholipids are important for determining and regulating the intermolecular arrangement (28). Although both phospholipids are zwitterionic, the relative packing areas of their polar-head groups with respect to the acyl chains, and thus their polarization, hydration, and mobility, differ (29, 30). As a consequence, the resultant polar-head group dipole-moment vectors lead to favorable matching of gangliosides with PtdCho,

but this is unfavorable for PtdEtn (31, 32). The interactions in the polar-head group region of lipid interfaces are generally dominant with respect to those occurring among the hydrocarbon chains (28, 32). This fact determines that differences in the polar-head group region markedly influence the intermolecular packing areas and, therefore, the phase state (7, 12). This situation is especially so for complex glycosphingolipids (12). Due to the amplification of this type of local molecular changes the overall membrane topology is very sensitive to what may be considered relatively small variations of lipid composition (27, 33). The variation of 2 mol % of the ganglioside composition in mixed monolayers with phospholipids can cause as much as 30–40% alteration of the intermolecular packing areas, depending on the surface pressure (27, 31). These changes are amplified through the critical packing parameter to modifications of the interfacial curvature, structural stability, and surface electrostatics (11, 14, 33, 34). We have previously shown by HSDSC that binary mixtures of dipalmitoylphosphatidylcholine containing a mole fraction of gangliosides <5 mol % exhibit markedly altered pretransition endotherm, and a defined amount of PtdCho is prevented from undergoing the main gel-to-bilayer phase transition (7). In ternary mixtures the favorable interactions of gangliosides with the bilayer-forming PtdCho, together with unfavorable ones with the H_{II}-forming PtdEtn, should facilitate formation of the hexagonal phase because relatively more PtdEtn is left uncompensated. In addition, this effect should only be expected at very low proportions of gangliosides because increasing the amount of such marked conically shaped molecules beyond a critical threshold would override local intermolecular effects and cause large alterations of the overall bilayer curvature (11, 12, 14, 33).

Several effects of gangliosides in biomembranes can be related to the results of the present work. Transient structures in H_{II}-phase configuration are important intermediates involved in hemi-fusion and whole fusion of bilayer membranes (35) triggered by a variety of lipids (4, 16) and proteins (36–38). Gangliosides and other neutral glycosphingolipids with long oligosaccharide chains reduce or inhibit the rate and extent of interactions and fusion induced by Ca^{2+} among bilayers containing phosphatidic acid and PtdEtn (39). Also, we reported that gangliosides inhibit both hemi-fusion and whole-bilayer fusion induced by myelin basic protein and mellitin (40), two proteins that cause formation of H_{II} phases (36, 37). The inhibition of bilayer fusion was more marked for gangliosides having longer and more hydrated (41) oligosaccharide chains and a smaller critical packing parameter (11, 14); this is also in keeping with the geometrical opposition of the ganglioside molecule to the geometrical requirements of the H_{II} phase.

Another correlation relevant to the present results is that moderate formation of H_{II} phase by a relatively small percentage of diacylglycerol enhances the activity of phospholipase A_2 and phospholipase C against phospholipid bilayers (42). We previously showed that gangliosides inhibit the rate of phospholipid hydrolysis by phospholipases A_2 and C acting not as classical inhibitors but through a surface-mediated mechanism (43, 44). The alteration of enzymatic activity was largely independent of the hydrocarbon chain and polar-head group moieties of the sphingolipid (45). The ganglioside regulation of phospholipase activity occurs beyond precatalytic interfacial steps and depends on the balance of several supramolecular factors influencing the phospholipid organization, phase state, hydration, and electrostatic field (43–45). In bulk dispersions with phospholipids gangliosides also show a dual effect on the rate of activity of phospholipase A_2 that depends on their mole fraction in the mixture, as well as on the phospholipid phase state (44). Thus, the ganglioside modulation of H_{II} phase formation shown in the present work is also in keeping with their effect on phospholipase activity. Our findings provide experimental basis for suggesting the possible participation of this type of supramolecular regulation by gangliosides of cellular processes mediated by membrane–membrane interactions and signal transduction in which these lipids have often been implicated as biomodulators (10, 46).

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