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The effect of gangliosides on the lamellar phase behaviour of phosphatidylethanolamines

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The thermotropic properties of aqueous phosphatidylethanolamine dispersions vary with hydration. Measured by EPR-spectroscopy freshly hydrated dimyristoylphosphatidylethanolamine dispersions exhibit a gel to liquid-crystalline phase transition at $T_{ml} = 48°C$. Dehydration could be induced by prolonged incubation of a hydrated sample at 4°C. The phase transition temperature of the dehydrated phase was determined to be $T_{mh} = 54°C$. From the measured phase transition curves we followed the dehydration with time and found a cooperative nucleation process. A 50% dehydration was reached after 5 days. This dehydration process could be prevented by gangliosides: 1.5 mol% of G_{T1b}, 4 mol% of G_{M1} or 7 mol% of G_{D1a} or G_{M3} but also 7 mol% of phosphatidic acid were able to stabilize the hydrated phase completely. The effect of gangliosides G_{M1}, G_{M3}, G_{D1a}, G_{T1b} and of the negatively charged phosphatidic acid on the phase behaviour of dimyristoylphosphatidylethanolamine (DMPE) dispersions were investigated. The phase transition temperature of freshly hydrated DMPE samples was successively decreased from 48 to 43°C with increasing amounts of G_{D1a} up to 10 mol% whereby the phase transition was significantly broadened. Gangliosides G_{M1}, G_{M3} and G_{T1b} as well as phosphatidic acid had minor effects. Dispersions of pure DMPE prepared below the transition temperature T_{ml} form the dehydrated phase again with a melting temperature of $T_{mh} = 54°C$. In the presence of 10 mol% G_{D1a} or G_{T1b} this value is reduced to T_{ml}, the phase transition temperature of the hydrated phase. The reduction induced by G_{M3} is less pronounced. With G_{M1} or phosphatidic acid the samples remain partially dehydrated and the phase transition curves become biphasic up to 7 mol% ganglioside or phosphatidic acid.

Introduction

The bilayer lipid membrane is the fundamental structural component and permeability barrier of biological membranes. Pure lipids have provided useful models for studying interactions of membrane lipids with other membrane components in reconstituted systems.

Abbreviations:

G_{M1},	monosialoganglioside G_{M1};
G_{M3},	monosialoganglioside G_{M3};
G_{D1},	disialoganglioside G_{D1a};
G_{T1b},	trisialoganglioside G_{T1b};
DMPA,	1,2-dimyristoyl-*sn*-glycero-3-phosphatidic acid;
DMPE,	1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine;
T_m,	main phase transition temperature for the gel to liquid-crystalline transition;
T_h,	phase transition temperature for the liquid-crystalline to hexagonal H_{II}-phase;
T_{ml},	phase transition temperature of the low melting hydrated gel phase;
T_{mh},	phase transition temperature of the high melting dehydrated subgel phase;
EPR,	electron paramagnetic resonance;
TEMPO,	2,2,6,6-tetramethylpiperidine-1-oxide.

Membrane phospholipids can be divided into different groups depending on their structural and physicochemical properties. One can distinguish cone-shape, and cylindrical inverted cone-shape types of lipids [1]. Phosphatidylethanolamines are major membrane phospholipids, belonging to the nonbilayer cone-shape type [2]. In this type of lipids the area of the head group is small compared to the area of the hydrocarbon chains and it is characterized by a high intra- and intermembrane hydrogen bonding capacity [3,4]. Therefore phosphatidylethanolamines form lamellar structures separated by unusually thin water layers. The water layer thickness is about 0.5 nm, which corresponds to the diameter of about two water molecules. Under certain conditions phosphatidylethanolamines prefer the nonbilayer inverted hexagonal (H_{II}) phase [5,6].

Other membrane phospholipid species like phosphatidylcholine, phosphatidylserine and phosphatidic acid readily form closed bilayer structures under physiological conditions. They belong to the bilayer forming cylindrical type of lipids due to the larger area occupied by the head group or due to electrostatic repulsion between neighbouring molecules [5,7].

Lipids, with a large volume relation between the head group and the hydrocarbon moiety belong to the inverted cone-shape type and are detergent like. Above a certain critical micellar concentration they organize themself in micelles. Well known examples are gangliosides, which form micelles at concentrations below 10^{-8} M [8]. Mixed cone-shape and inverted cone-shape lipids, e.g. phosphatidylethanolamines and gangliosides, can pack complementarily to form bilayer structures.

The structural polymorphism of phosphatidylethanolamines depends on temperature, hydration and lipid chain length [4].

Two solid bilayer phases that differ in their hydration have been described [9,10]. Both phases show a bilayer to bilayer type phase transition. In hydrated samples the well known gel phase (L_β) with hexagonal chain packing is metastable and converts into the $L_{\beta'}$ phase. The phase transition to the L_α phase is observable at T_m.

A second stable crystalline or subgel bilayer-phase with orthorhombic acyl chain arrangement was described, which directly converts to the liquid-crystalline phase at a temperature much higher than T_m [9,11,12]. This stable solid phase can be obtained by long time incubation of a hydrated sample at low temperature (4° C) or by sample preparation at $T < T_m$ [10]. This phase is characterized by low hydration, tight interchain packing and by an irreversible high-temperature phase transition [6,13].

At a temperature $T_h > T_m$ the L_α phase converts into the H_{II} phase which is accompanied by additional conformational and motional disorder due to an increased number of gauche conformers in the acyl chain region near the lipid head group [13–17]. This phase will not be considered in the present work.

Gangliosides as well as phosphatidylethanolamines are enriched in nerve cell membranes, where their function and interaction is far from being understood. It has been supposed that gangliosides serve as Ca^{2+}-storage agents and regulate nerve cell membrane permeability [18,19]. However, very recently we have shown that gangliosides bind Ca^{2+} with low affinity comparable to phosphatidylcholines [8] which excludes a preferential Ca^{2+}-binding. In extraneuronal tissue gangliosides have been implicated in various surface recognition and physiological events [20,21].

The membrane function of gangliosides may well depend on the interaction with membrane lipids which are able to form hydrogen bonds like ethanolamines. Therefore we studied mixed lipid systems of phosphatidylethanolamine and gangliosides in the lamellar phases. The aim was to characterize the hydration and dehydration behaviour of phosphatidylethanolamine membranes in the presence of physiological amounts of gangliosides.

Part of this work has been presented on the 3rd European Workshop on Bacterial Protein Toxins, Überlingen, 1987.

Material and Methods

Analytical grade dimyristoylphosphatidylethanolamine (DMPE) was purchased from Fluka (Neu-Ulm, F.R.G.), stored at −20° C, and used as long as no degradation product (e.g. lyso-phosphatidylethanolamine) could be found on analyti-

cal TLC. 2,2,6,6-Tetramethylpiperidine-1-oxide (TEMPO) was from Aldrich (Steinheim, F.R.G.).

Ganglioside extraction

Gangliosides were extracted from bovine brain gray matter with chloroform/methanol/water followed by phase partition according to Svennerholm and Fredman [22]. The crude ganglioside extract was purified by ion exchange chromatography on DEAE-sepharose [23]. Pure G_{M1} and G_{T1b} as well as a mixture of disialogangliosides were obtained. The latter were further purified by adsorption chromatography on an iatrobeads column [24], which separated G_{D1a} and G_{D1b}. The purity of the isolated gangliosides was analysed by TLC on high performance silica gel coated plates [25] and sialic acid determination [26].

Vesicle preparation

Gangliosides and phospholipids were dissolved in chloroform/methanol (1 : 1, v/v) and mixed in the appropriate amounts in glass tubes. Dry lipid films of DMPE and DMPE/ganglioside could be obtained by evaporation of the organic solvent by a stream of nitrogen at $T > T_m$ and keeping the sample under vacuum for two hours.

Pure DMPE lipid films were dispersed by 5 min sonication (Branson sonifier, microtip, power level 20 W) in 10 mM Tris-HCl buffer (pH 7.2) containing 10^{-3} M TEMPO spinlabel. The final lipid concentration was 1.6 mg/ml. Mixed DMPE/ganglioside films were dispersed in the same buffer but were vortexed with glass beads at a final lipid concentration of 4 mg/ml. It was necessary to use different dispersion methods for pure and mixed lipid samples, because DMPE hardly forms homogeneous dispersions in the aqueous phase. In the presence of gangliosides the lipids easily formed vesicle structures as was checked by electron microscopy (data not shown).

Hydrated and dehydrated samples were obtained according to Mulukutla and Shipley [9] and Chowdhry and Dalziel [2]. Hydrated samples were dispersed above T_m at 60 °C, quickly cooled to 2 °C in icewater and immediately used for EPR spectroscopy. Hydrated preincubated samples were dispersed at 60 °C, slowly cooled to 4 °C in a refrigerator and stored at this temperature for varying times (1 day to 2 weeks). Dehydrated samples were prepared by dispersion of the lipid films in the above mentioned buffer at 15 °C followed by 4 days incubation at 4 °C. All samples were sedimented at $12000 \times g$ (Hettich microcentrifuge) and the pelleted liposomes were transferred into quartz capillaries for EPR measurements. Long time incubation did not cause phospholipid degradation as was shown by TLC.

Analytical procedure

Incorporation of ganglioside into the DMPE lipid phase was controlled by analytical determination of phosphorus [27] and sialic acid [26] in the sedimented liposomes. Ganglioside concentration is given in mol% with respect to total lipid.

EPR–TEMPO-partitioning

Phase transition temperatures were determined by measuring the TEMPO-partitioning between the aqueous and the lipid phase [28]. EPR spectra were taken with a Varian E-4 spectrometer. Temperature was regulated by a heated N_2-flow and controlled by a Keithley digital thermometer.

Results

Formation of the dehydrated phase in pure DMPE bilayers

Previous studies using differential scanning calorimetry have shown that aqueous dispersions of DMPE can form two quite different types of solid phases in excess water [9–11]. A fully hydrated gel phase forms rapidly by preparing the sample above the crystalline to liquid-crystalline phase transition temperature T_m. The dehydrated phase can be generated either by dispersing the lipid in the aqueous medium below T_{ml} or by incubating an initially hydrated sample at low temperature (4 °C) for long periods of time. Fig. 1 shows the phase transition curves of a hydrated and a dehydrated DMPE sample obtained by EPR-spectroscopy. The hydrated phase melts at $T_{ml} = 48$ °C and the dehydrated phase at $T_{mh} = 54$ °C. These values of the phase transition temperature are slightly below those ($T_{ml} = 49.6$ °C and $T_{mh} = 56.3$ °C) observed by Wilkinson and Nagle [11] using calorimetry.

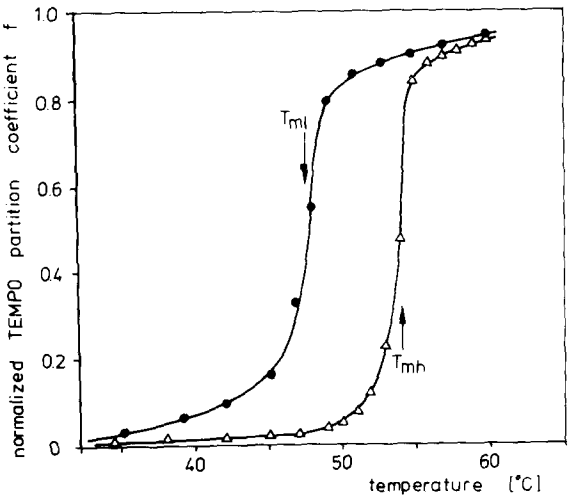

Fig. 1. Phase transition curves of a freshly hydrated (●———●) and a dehydrated (△———△) DMPE sample measured by EPR spectroscopy using the TEMPO-partitioning method. The midpoints of the melting curves were taken as phase transition temperatures, which we determined to be $T_{ml} = 48\,^{\circ}C$ and $T_{mh} = 54\,^{\circ}C$ for the hydrated and dehydrated sample, respectively. Lipid dispersions were prepared by sonication at a concentration of 1.6 mg/ml in 10 mM Tris-HCl buffer (pH 7.2) at 60 °C for the hydrated probe and at 15 °C for the dehydrated probe. Hydrated probes were quickly cooled in icewater and were immediately used for measurement. Dehydrated samples were incubated for 4 days at 4 °C before measurement. Scans were taken with increasing temperature.

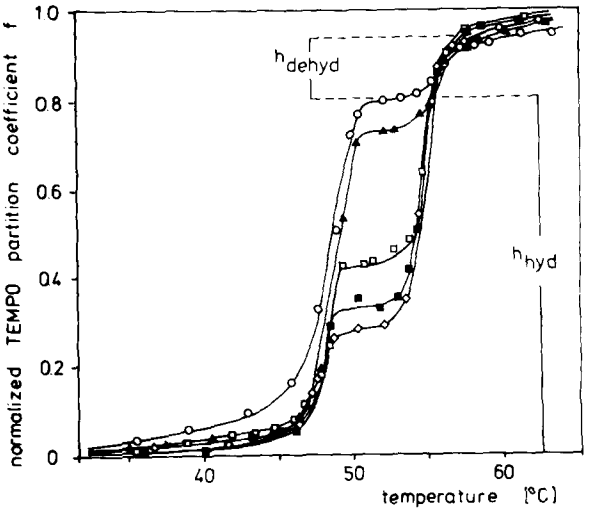

Fig. 2. Phase transition curves for hydrated DMPE samples, which were incubated at 4 °C for varying times. The incubation times were: 1 day (○———○), 4 days (▲———▲), 5 days (□———□), 6 days (■———■) and 11 days (◇———◇), respectively. Samples were prepared at 60 °C by sonication at a lipid concentration of 1.6 mg/ml, slowly cooled to 4 °C and incubated at that temperature for the given time. The step heights h_{hyd} and h_{dehyd} of the corresponding phase transitions are marked for a sample after one day of incubation.

We investigated the time dependence of the dehydration process. Initially hydrated samples were incubated at 4 °C for varying times up to eleven days in order to induce the transformation from a hydrated to the dehydrated PE phase. Phase transition curves as shown in Fig. 2 were taken starting after one day of incubation. The obtained two step phase transition curves clearly demonstrate the coexistence of a hydrated and a dehydrated phase. From the corresponding step height we were able to estimate the amount of lipid in either phase. Until the fourth day of incubation the sample remains almost completely in the hydrated state. Between the fourth and fifth day a considerable amount of lipid converts into the dehydrated form. This dehydration process finally reaches a value of 70% after 7 days or longer. A complete relaxation into the stable dehydrated state could not be reached within two weeks which is in agreement with earlier studies of Mulukutla and Shipley [9]. The time course of the dehydration process is shown in Fig. 3. The fraction of the dehydrated phase was calculated from the height of the corresponding phase transition step (h_{dehyd}) with respect to the total height

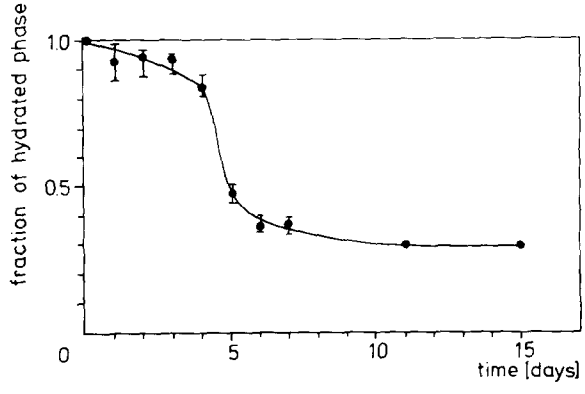

Fig. 3. Time dependence of the dehydration after incubation at 4 °C of originally hydrated samples. The fraction of the hydrated phase was determined from the quotient $h_{hyd}/(h_{hyd} + h_{dehyd})$ of the corresponding step heights as shown in Fig. 2.

($h_{dehyd} + h_{hyd}$) of the phase transition which is normalized to one in Fig. 2. The obtained time dependence has a sigmoidal shape.

The dehydrated phase immediately converts to the hydrated phase if the sample is once heated above it's phase transition temperature T_{mh}. Rescanning a dehydrated sample with decreasing temperature yields a phase transition curve with $T_{ml} = 48°C$. This indicates, that conversion from a solid L_β phase to the fluid L_α phase is accompanied by a spontaneous and complete hydration of PE samples.

The effect of gangliosides on the phase transition temperature of hydrated and dehydrated DMPE phases

The main emphasis of the present study was to investigate the effect of gangliosides on the different DMPE phases and on their formation. Mixed membranes containing DMPE and low concentrations (1–10 mol%) of gangliosides G_{M1}, G_{M3}, G_{D1a} and G_{T1b} were prepared under the above mentioned conditions. Phosphatidic acid (DMPA) was used for comparison to determine the pure charge effect. In contrast to gangliosides phosphatidic acid carries its negative charge in a fixed position at the membrane surface and therefore we can exclude an interference with different headgroup conformations.

First we investigated the effect of G_{M1}, G_{M3}, G_{D1a}, G_{T1b} and of phosphatidic acid on the hydrated DMPE phase. 1–10 mol% of G_{M1}, G_{M3}, G_{T1b} or phosphatidic acid have no significant effects on the phase transition of hydrated DMPE. T_{ml} is shifted within + 1 C° and the transition becomes slightly less cooperative at increasing ganglioside concentrations (data not shown).

Remarkably different results are obtained with G_{D1a} (Fig. 4). The DMPE phase transition is continuously shifted downward from 48°C to 43°C by the incorporation of 1–10 mol% of the disialoganglioside and the transition curve is significantly broadened. Obviously G_{D1a}, but not G_{M1}, G_{M3} or G_{T1b}, destabilizes the hydrated phosphatidylethanolamine matrix and alters the lipid pack order.

Secondly we investigated the influence of the different gangliosides on dehydrated DMPE membranes (Figs. 5a and b). Samples were prepared at

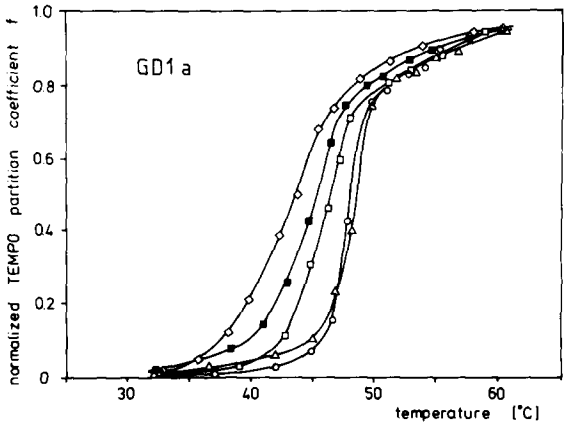

Fig. 4. Phase transition curves of freshly hydrated mixed DMPE/G_{D1a} membranes. Ganglioside concentrations with respect to total lipid were: 1 mol% (○———○), 3 mol% (△———△), 5 mol% (□———□), 7 mol% (■———■) and 10 mol% (◇———◇), respectively. Samples were prepared at 60°C by vortexing with glass beads at a lipid concentration of 4 mg/ml in buffer, quickly cooled in icewater and were immediately taken for measurement.

$T < T_m$ and in addition were hold at 4°C for 4 days. The dehydrated phase is formed in pure PE under these conditions.

From the phase transition curves we realize an almost unchanged dehydrated phase at 1 mol% concentration of ganglioside G_{M1}. The transition curves of DMPE membranes containing G_{D1a} or G_{T1b} are slightly broadened at that concentration.

Differences are found at higher ganglioside concentrations. Monosialoganglioside G_{M1} up to 4 mol% causes a biphasic transition curve with a low melting component between 45°C and 52°C and a highly cooperative melting component at 54°C. A broad but almost monophasic transition with a transition temperature of about 51°C was found in the presence of 5 mol% G_{M1}. Increasing the G_{M1} content to 10 mol% further decreases the phase transition temperature by only one degree but the cooperativity of the transition remains unchanged. Ganglioside G_{M3} reduces T_{mh} to about 50°C up to 10 mol% concentration but the transition curves remain monophasic (data not shown).

G_{D1a} (Fig. 5b) at 3 and 6 mol% concentration remarkably inhibits the formation of the dehydrated phase. In the presence of 10 mol% G_{D1a} we obtained a phase transition curve that is almost identical to the one obtained for pure hydrated

DMPE (Fig. 1). Comparable phase transition curves were found with G_{T1b}.

The phase transition curves obtained for mixed DMPE/DMPA membranes prepared under dehydrating conditions are shown in Fig. 5c. In the presence of 3 or 5 mol% DMPA the phase transition curve is biphasic and similar to the one obtained in the presence of G_{M1}. DMPA as well inhibits the formation of the hydrated phase but to a smaller extent.

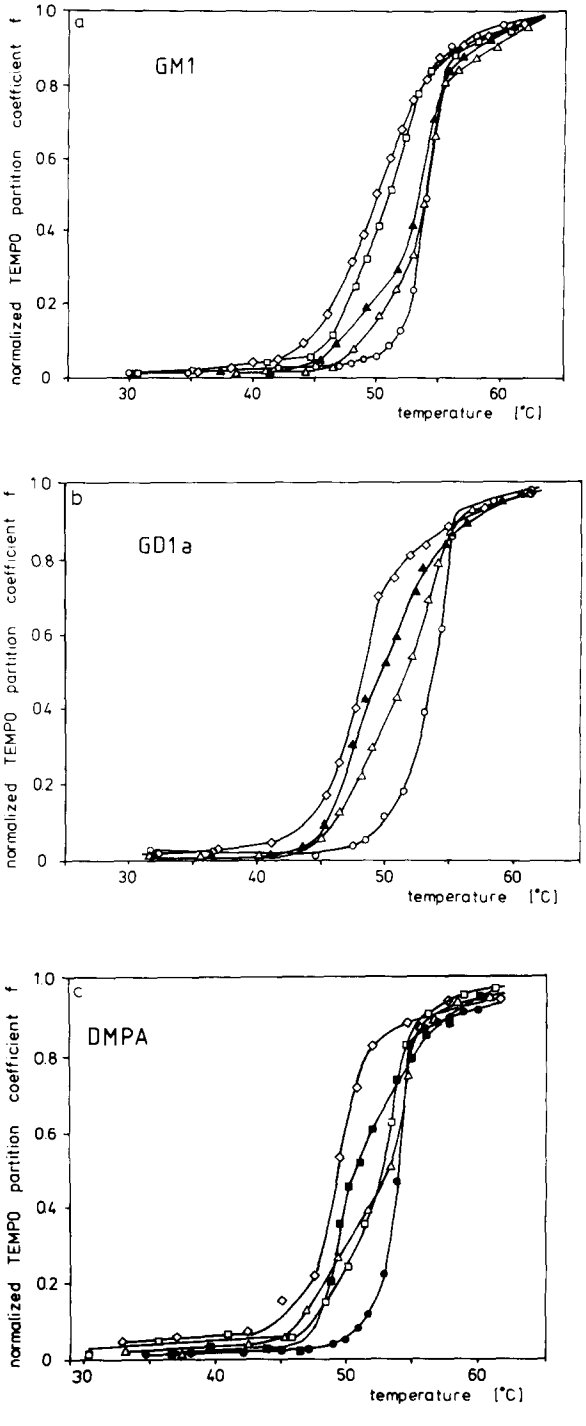

Fig. 5. Phase transition curves of mixed DMPE/ganglioside membranes under dehydrating conditions. Dispersions were prepared below the transition temperature at 15 °C by vortexing with glass beads at a final concentration of 4 mg/ml in buffer. Samples were held at 4 °C for 4 days before measurement. (a) DMPE/G_{M1}: 1 mol% (○——○), 3 mol% (△——△), 4 mol% (▲——▲), 5 mol% (□——□) and 10 mol% (◇——◇), respectively. (b) DMPE/G_{D1a}: 1 mol% (○——○), 3 mol% (△——△), 6 mol% (▲——▲), 10 mol% (◇——◇) ganglioside. (c) DMPE/DMPA: 1 mol% (●——●), 3 mol% (△——△), 5 mol% (□——□), 7 mol% (■——■) and 10 mol% (◇——◇) DMPA.

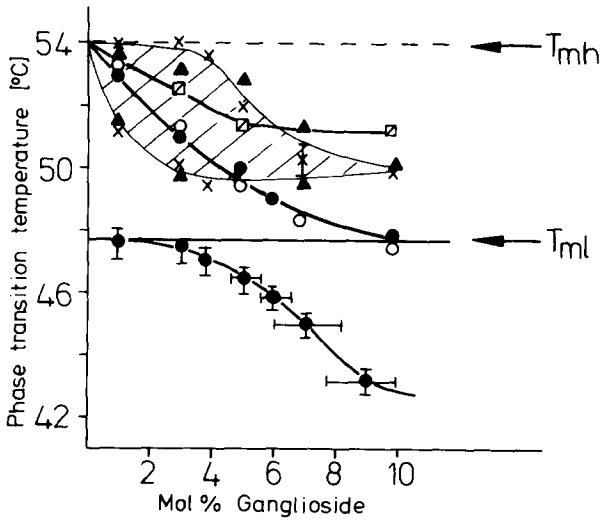

Fig. 6. Change in phase transition temperature of dehydrated (T_{mh}) and hydrated (T_{ml}) DMPE-membranes with ganglioside concentration. Values for T_{mh} were taken from Fig. 5. Biphasic phase transition curves are considered by an upper and a lower transition midpoint. T_{ml} values for G_{D1a} were taken from Fig. 4. Other gangliosides do not alter T_{ml} remarkably as is shown by the straight line. G_{D1a} (●——●), G_{T1b} (○——○), G_{M3} (⊠——⊠), G_{M1} (×——×), DMPA (▲——▲).

The effect of gangliosides G_{M1}, G_{M3}, G_{D1a} and G_{T1b} on the phase transition temperatures of hydrated and dehydrated PE-bilayers is summarized in Fig. 6. In the presence of G_{D1a} and G_{T1b} the phase transition of the dehydrated PE-phase is reduced from T_{mh} to T_{ml}. With G_{M1} we observed a

biphasic transition up to 5 mol% (hatched area). Even in the presence of 10 mol% T_{mh} is only reduced to 50° C. Equivalent results were obtained for phosphatidic acid in PE-membranes prepared under dehydrating conditions. G_{M3} reduces T_{mh} to 51° C.

Hydrated PE-membranes are not significantly effected by G_{M1}, G_{M3}, G_{T1b} or phosphatidic acid. G_{M1} and G_{M3} slightly increases the phase transition temperature by 0.5 C°. In contrast G_{D1a} is the only ganglioside in our series that reduces T_{ml} from 48 to 43° C with increasing concentration.

A next set of experiments was designed to examine the stability of the hydrated gel phase during incubation at 4° C in the presence of gangliosides. As was shown in Figs. 2 and 3 the pure DMPE converts to the high-melting solid phase under these conditions. We chose an incubation time of 5 days, after which the pure PE host lipid shows 50% dehydration. A typical result is shown in Fig. 7. In the absence of ganglioside the phase transition curve shows the coexistence of the hydrated and dehydrated phases in a clearly separated two step image. The midpoints of the transitions again are considered to be T_{ml} and T_{mh}, respectively. The step height is taken to be proportional to the amount of lipid in the appropriate phase.

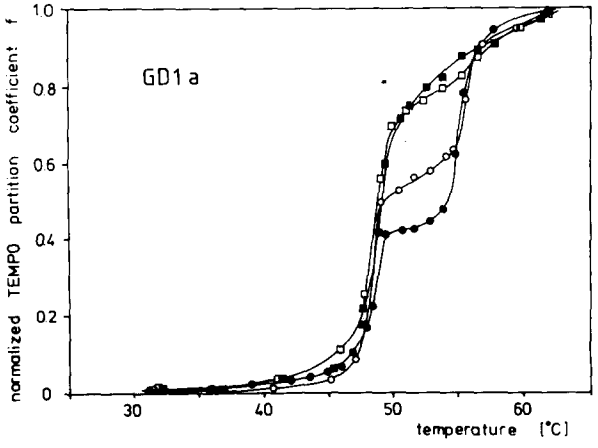

Fig. 7. Phase transition curves of initially hydrated mixed DMPE/G_{D1a} samples, which were incubated at 4° C. Samples were prepared at 60° C by vortexing with glass beads, slowly cooled to 4° C and incubated at that temperature for 5 days. The lipid concentration was 4 mg/ml. The corresponding phase transition of pure DMPE is given as reference (●——●). Ganglioside concentrations were: 1 mol% (○——○); 5 mol% (□——□) and 7 mol% (■——■) with respect to total lipid.

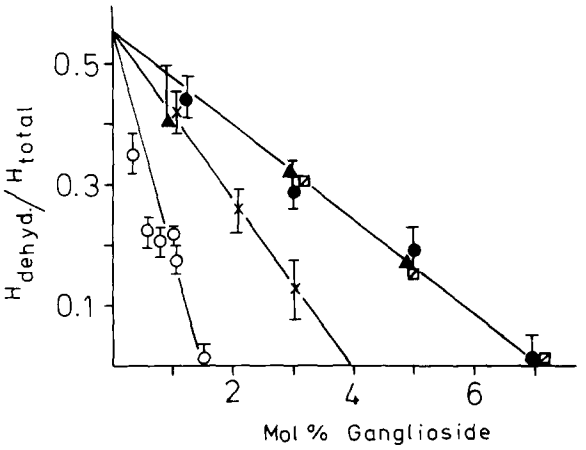

Fig. 8. The fraction of PE in the dehydrated phase as determined from the 'two step' curves is given as function of ganglioside concentration. The extrapolations of the obtained straight lines yield the minimal amount of ganglioside necessary to inhibit the formation of the dehydrated phase. G_{T1b} (○——○), G_{M1} (×——×), G_{M3} (☑——☑), G_{D1a} (●——●), DMPA (▲——▲).

In the presence of G_{D1a} (and also of G_{M1}, G_{T1b} and phosphatidic acid) the step height of the transition assigned to the dehydrated phase decreases with increasing ganglioside concentration and diminishes at 7 mol% in the case of G_{D1a}. It is important to note that the hydrated phase is not affected by G_{D1a} even at 7 mol%.

Similar experiments performed with the other gangliosides and phosphatidic acid are documented in Fig. 8. We used the step height ratio of the phase transition curves to determine the ganglioside concentration which is necessary to stabilize the initial hydration state. The phase transition of the dehydrated phase has vanished at concentrations of 7 mol% G_{D1a}, G_{M3} or phosphatidic acid, at 4 mol% G_{M1} and already at 1.5 mol% G_{T1b}.

Discussion

This EPR-spectroscopic study is aimed at the understanding of ganglioside–phospholipid interaction. We have verified previous calorimetric data for pure DMPE and have shown for the first time that dehydration is a cooperative nucleation pro-

cess. Our results clearly indicate, that gangliosides stabilize the fully hydrated state of DMPE membranes under dehydrating conditions and at concentrations comparable to the physiological situation.

It is generally accepted that membrane phospholipids possess a hydration shell in which water molecules are organized at the polar head group region and form a water layer between adjacent lipid bilayers. Removal of this water-shell is known to have important consequences for the physical state of the lipids. Chapman et al. [29] demonstrated that the main phase transition temperature of phosphatidylcholine increases by about 30 °C when the lipid is dehydrated. More recently X-ray diffraction [3,4,16] and calorimetric data [9,11] exhibited an inverted relation between the grade of hydration and the lipid phase transition temperature. The interpretations of our phase transition curves obtained by EPR spectroscopy are based on these calorimetric data, since spin labels do not provide direct evidence for a hydration or dehydration. In our hands dehydration starts in a cooperative way. This means that the first contact between approaching lipid bilayers is the activation step. If the hydration shell is removed in this contact zone dehydration spreads over a long distance.

Further we have shown that oligosaccharide head group structures, which together with glycoproteins form the eucaryotic glycocalix, influence the structural properties of their phospholipid environment.

With fully hydrated DMPE we obtained one highly cooperative gel to liquid-crystalline transition at $T_{ml} = 48$ °C (Fig. 1). Gangliosides G_{M1}, G_{M3} and G_{T1b} had a minor effect on this transition, indicating a complete protrusion of the head groups into the interspace water. We conclude that the interaction between the hydrated phospholipid and ganglioside head groups is negligible. Ganglioside G_{D1a} promoted a significant fluidisation of the host lipid membrane, indicated by the decrease in the T_{ml} values with increasing ganglioside concentration (cf. Fig. 4). This effect is most likely caused by phospholipid-ganglioside head group interaction, leading to reduced hydrogen bonding between the phosphatidylethanolamine head groups and to an increased overall hydration. Such an effect was not observed in phosphatidylcholine membranes where all gangliosides induce an increase in phase transition temperature (Ollmann and Galla, unpublished results). Therefore possible differences in fatty acid composition of the gangliosides are not expected to contribute to the observed differences in PE membranes. This may also be concluded from a recent study of Masserini and Freire [30] who showed that the thermotropic behaviour of phosphatidylcholine vesicles containing ganglioside G_{M1} is independent of ganglioside chain length composition.

Tsao et al. [31] performed a thermodynamic and phase characterization of PE-ganglioside G_{D1a} mixtures. They observed an increase in the lipid phase transition temperature with increasing ganglioside content. Moreover, the authors postulate the formation of semi-fluid PE-G_{D1a} complexes in fluid PE-domains. Both results seem to be conflicting with our present and an earlier excimer fluorescence study [8]. However, Tsao et al. [31] used PE with unsaturated lipid chains which may behave different from our saturated dimyristoylphosphatidylethanolamine membranes.

We propose that the headgroup of G_{D1a} exhibits a conformation where the sialic acid residues are directed to the membrane surface and disturb the hydrogen bonds between the ammonium group and the phosphate group of neighbouring PE molecules. This hydrogen bond network stabilizes the pure PE layer. The carboxylic groups of the sialic acids function as hydrogen bond acceptors and may therefore invade the surface region thus expanding the PE layer. Consequently the phase transition temperature will be reduced. The fact that this is only induced by the disialoganglioside but not by monosialo- or trisialogangliosides raises the possibility that only G_{D1a} assumes a conformation which allows the incorporation of the sialic acid residue into the PE-headgroup region. This is only possible if the oligosaccharide part of G_{D1a} is bent back to the membrane surface whereas with G_{M1}, G_{M3} or G_{T1b} it protrudes out of the membrane into the aqueous space. The reduced cooperativity of the phase transition step is probably due to disordering in the membrane interior by the incorporated ceramide moiety of the gangliosides.

The dehydrated high-melting solid phase of ethanolamines is characterized by tighter interchain packing, immobilization of the lipid head groups and a very short lamellar repeat distance, that suggests a virtual absence of water between the lipid lamellae [13,32]. Seddon et al. [6] found by X-ray diffraction that there is one water molecule per lipid bound in the high melting solid phase of the ether linked didodecylPE. The chain-melting temperature begins to increase when the water content falls below six water molecules/lipid.

We obtained the dehydrated solid phase by preparing the sample below T_m, at 15°C. This condition of preparation leads to a dense chain and head group packing and to an initially reduced water space [10,13]. Additional incubation at low temperature (4°C) for at least 12 hours is not necessary for the formation, but for stabilization of the orthorhombic chain packing arrangement [9]. With these dehydrated samples we could clearly demonstrate that gangliosides with exposed carbohydrate structures are able to maintain a certain hydration state or to prevent a dehydration of the DMPE matrix.

G_{D1a} and G_{T1b} reduce the phase transition temperature T_{mh} to the value T_{ml} of the hydrated PE-phase. Presumably these two gangliosides act as a spacer between bilayer membranes and thus stabilize the hydrated PE-phase. Unfortunately we did not succeed in preparing and purifying vesicles with a G_{D1a} content exceeding 10 mol% because above that concentration mixed phospholipid/ganglioside micelles are formed remaining in the supernatant of the centrifuged samples. Thus we were not able to check if higher G_{D1a} contents further decrease T_{ml} as was observed in hydrated PE.

With G_{M1} and phosphatidic acid in PE-membranes we observed a biphasic phase transition in a concentration range between 2 and 7 mol%. This must be interpreted as a tendency to phase separate. A more hydrated phase possibly containing higher amounts of G_{M1} or phosphatidic acid coexists with a dehydrated phase. This is not surprising with phosphatidic acid which has a high tendency to phase separate even in fluid bilayer membranes [33]. However, it is surprising that G_{M1} undergoes a phase separation but not G_{M3} that carries the same charge but has a smaller head group. In an earlier paper using pyrene labeled gangliosides we observed that PyG_{M3} has a stronger preference for a fluid phosphatidylcholine membrane than PyG_{M1} [8]. We assume that G_{M3} may also have a preference for fluid PE-domains, i.e., for already hydrated regions.

Finally, the experiments with hydrated preincubated samples, which were obtained by keeping the samples at 4°C for 5 days, thus leading to a partial dehydration in pure PE-membranes, are worth discussing. Again gangliosides as well as DMPA prevent dehydration (Figs. 7 and 8). This is demonstrated by the stepwise disappearance of the high-melting phase. Most likely the dehydrated phase forms in these initially hydrated samples by squeezing out the gangliosides from the dehydrated regions. This is indicated by the sharp high-temperature transition. At high concentrations the probe molecules cannot be further separated and the sample remains in the fully hydrated state with homogeneous probe distribution.

Gangliosides may act differently in the process of stabilization of a hydrated phase and in rehydration of a dehydrated phase. These two processes seem to need different modes of structural reorganisation, namely by forming nucleation centers for dehydration and, on the other hand preventing the orthorhombic chain packing with reduced water space. G_{T1b} was found to be most effective in the stabilization of the hydrated phase followed by G_{M1}. G_{M3}, DMPA and G_{D1a} are less effective. These differences are most likely due to probe distribution and head group conformation.

It was found by X-ray diffraction that the G_{M1} head group extends at least 2.1 nm from the hydrocarbon/water interface [32]. The fixed charge is located away from the bilayer surface and therefore the surface potential is decreased compared to charged phospholipids. A headgroup structure like this obviously increases the water layer thickness of the otherwise densely packed DMPE. This explains the ability of G_{M1} to stabilize the hydrated phase. The same effect is brought about by the space filling highly charged head group of G_{T1b}.

The G_{D1a} head group is believed to be inclined to the membrane surface, wrapping the phos-

pholipid head groups [35]. By this conformation one can imagine that there is no significant increase in lamellar repeat, i.e. increased water space, but a higher hydration in the headgroup region leading to less dense packing and decreased phase transition temperatures. The G_{M3} head group may be too small to act as a spacer and pure charge-charge repulsion comparable to the one induced by phosphatidic acid prevents dehydration only at high contents of the negatively charged lipid.

We conclude that the G_{M1} and G_{T1b} head groups protrude out into the water phase, thus acting as a hydrated spacer between adjacent PE-bilayer membranes. G_{T1b} which has the largest head group and in addition is most highly charged has the strongest potency to keep a membrane surface hydrated.

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