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Direct visualization of the lateral structure of giant vesicles composed of pseudo-binary mixtures of sulfatide, asialo-GM1 and GM1 with POPC

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ABSTRACT

We compared the lateral structure of giant unilamellar vesicles (GUVs) composed of three pseudo binary mixtures of different glycosphingolipid (GSL), i.e. sulfatide, asialo-GM1 or GM1, with POPC. These sphingolipids possess similar hydrophobic residues but differ in the size and charge of their polar head group. Fluorescence microscopy experiments using LAURDAN and $DiIC_{18}$ show coexistence of micron sized domains in a molar fraction range that depends on the nature of the GSLs. In all cases, experiments with LAURDAN show that the membrane lateral structure resembles the coexistence of solid ordered and liquid disordered phases. Notably, the overall extent of hydration measured by LAURDAN between the solid ordered and liquid disordered membrane regions show marked similarities and are independent of the size of the GSL polar head group. In addition, the maximum amount of GSL incorporated in the POPC bilayer exhibits a strong dependence on the size of the GSL polar head group following the order sulfatide > asialo-GM1 > GM1. This observation is in full harmony with previous experiments and theoretical predictions for mixtures of these GSL with glycerophospholipids. Finally, compared with previous results reported in GUVs composed of mixtures of POPC with the sphingolipids cerebroside and ceramide, we observed distinctive curvature effects at particular molar fraction regimes in the different mixtures. This suggests a pronounced effect of these GSL on the spontaneous curvature of the bilayer. This observation may be relevant in a biological context, particularly in connection with the highly curved structures found in neural cells.

1. Introduction

Glycosphingolipids (GSLs) are ubiquitous components of animal cell membranes [1]. They participate in a wide variety of physiologically relevant phenomena, including cell recognition and signal transduction processes [1,2]. For instance, GSLs containing simple polar head groups are known to stabilize membranes, possibly through inter-lipid hydrogen bonds [3–5]. Neutral and charged GSLs occur in relatively large amounts in specialized membranes in the nervous system such as myelin [6–9]. At present the effects of these lipids on the structure and function of cell membranes remain unclear.

Gangliosides are enriched in nerve cells where they represent between 2% and 10% of total lipids, contributing approximately 30% of the sialic acid content of the neuronal surface [1,5]. Gangliosides contain one or more sialic acid residues attached to a neutral oligosaccharide chain resulting in a series of polar head-groups of different complexity. The hydrophilic portion of these gangliosides reaches a length similar to that of the hydrocarbon portion and, in turn, dramatically influences the surface, thermotropic and topological properties of membranes containing them [1]. In addition, the number and type of carbohydrates in the polar head-group of these GSLs also have a direct influence on the extent of the interfacial hydration or micropolarity of GSL containing membranes [10–14].

Previous work has elucidated relevant biophysical properties of a series of chemically related sphingolipids ranging from sphingosine, ceramide, cerebrosides and sulfatides to complex gangliosides [1,15–17]. In particular, spatially resolved information about the lateral structure of membranes containing some sphingolipids have been reported, both in model systems and natural membranes by performing fluorescence microscopy experiments in giant unilamellar vesicles (GUVs) [18–24]. The most studied sphingolipid using this approach is sphingomyelin, and several papers using GUVs have been reported in the context of liquid immiscibility directly observed in membranes composed of sphingomyelin/DOPC/cholesterol [19,25–27]. In some of

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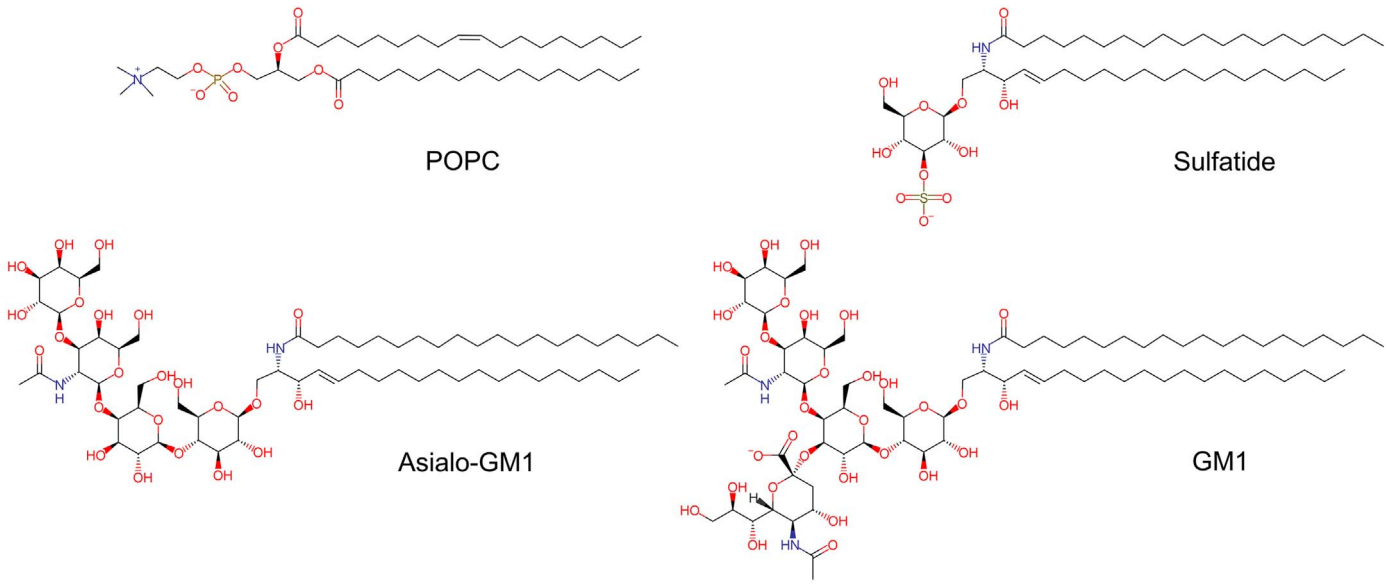

Fig. 1. Molecular structure of Sulfatide, asialo-GM1 (Gg4Cer) and GM1. The molecular structure of POPC is included for comparative purposes (see text).

these studies the ganglioside GM1 has been incorporated in lower proportions to these ternary mixtures mainly as a marker for the liquid ordered phase (using it as a receptor for fluorescently labeled cholera toxin; see for example [27]). However, few studies using microscopy techniques (fluorescence or atomic force microscopy) have addressed about the direct influence of GM1 (or asialo-GM1) on the phase coexistence scenario displayed by this ternary mixture [28–31] or in mixtures with POPC [32].

In this report we study in a comparative manner spatially resolved information obtained by incorporating different proportions of distinct GLS, i.e. GM1, asialo-GM1 (Gg_4Cer) and sulfatide (Fig. 1), in GUVs containing POPC. LAURDAN GP and $DiIC_{18}$ laser scanning confocal fluorescence imaging (one or two photon excitation) are used as the main experimental tool to explore these systems. Our results show a marked effect of these GLSs not only on the lateral structure of the POPC liquid disordered membrane but also on the whole structure of the lamellar system.

2. Materials and methods

2.1. Materials

LAURDAN and $DiIC_{18}$ were purchased from Invitrogen, Denmark. POPC was purchased from Avanti Lipids, Inc. (Alabaster, AL). Lactone-free GM1 from bovine brain was prepared according to Fidelio et al., 1991 [33]. Asialo-GM1 was prepared from the parent GM1 by acid hydrolysis in the presence of DMSO and purified as described by Rodriguez et al., 1996 [34]. Bovine brain sulfatide (3-O-sulfogalactosylceramide) was prepared and purified following the method of Wells & Dittmer 1965 [35]. Substitution of the N-acyl chain by stearic acid was carried out according to Kopaczyc & Radin, 1965 and Carter et al., 1981 [36,37]. HPTLC of each purified lipid in amounts at least 10 times that required for detection showed no contaminant spots after charring the plate with 50% (v/v) H_2SO_4. The composition of the hydrocarbon moiety of each preparation, as determined by GC and HPLC, was as follows: GM1 and asialo-GM1: the sphingoid base was constituted by C18:1-Sphingosine (79.7%), C20:1-Sphingosine (15.6%), C18:1-Sphinganine (4.5%); their N-acyl chain was constituted by C18:0 (95%), C16:0 (2%), C20:0 (1%), C22:0 (1.5%). Sulfatide: the sphingoid base was composed of C18:1-Sphingosine (81.7%), C20:1-Sphingosine (12.6%), C18:1-Sphinganine (2.5%). The N-acyl chain was constituted by C18:0 (90.5%), C24:0 (3%), h-C24:0 (2.5%), C:22:0 (1.6%), C 20:0 (1.8%). To avoid confusions and since each of the glycosphingolipids used in this paper present heterogeneous composition in their hydrophobic moiety, we decided to name their mixtures with POPC as pseudo-binary mixtures.

2.2. Preparation and quantification of lipid stock solutions

GM1 stock solution was prepared in chloroform-methanol-0.01 M NaOH (60:30:4.5) [33] and quantified by the resorcinol/HCl method [38] using Neu5Ac as the reference standards (Sigma Chemical Co.). Similarly, asialo-GM1 stock solution was prepared in chloroform-methanol-0.01 M NaOH (60:30:4.5) and determined by the anthrone-sulfuric acid method [39]. Sulfatide stock solution was prepared in chloroform-methanol-water (2:1:0.1) and quantified according to [40], a colorimetric assay based on the formation of colored salts with methylene blue that are extractable into chloroform. POPC stock in $CHCl_3$ was quantified using phosphorus analysis [41]. Stock solutions for the different pseudo-binary mixtures (0.2 mg/ml) were prepared in chloroform-methanol 2:1 by mixing appropriate aliquots of the different lipid stocks including the fluorescent probes. Probes were 0.1 and 1 mol% with respect to total lipids for DiIC18 and LAURDAN, respectively.

2.2.1. Preparation of GUVs

GUVs were prepared using the electroformation method reported by Angelova et al. [42] using a particular protocol reported elsewhere [20]. Briefly, 3 μl of the lipid mixture's stock solution was spread onto each platinum wire of a special custom built chamber [20] and the organic solvent evaporated using a stream of N_2. After this step, the chamber was placed under vacuum for at least 2 h to remove residual organic solvent. Subsequently, the chamber was assembled and the lipid films hydrated at 70 °C for 15 min using a 0.2 M sucrose solution in presence of an alternate electric field (Amplitude = 2 V and frequency = 10 Hz). The electric field was applied using a function generator (FG100 Vann Draper DigimessFg 100, Stenson, Derby, UK). After this procedure the frequency of the electric field was lowered to 1 Hz for 15 min, in order to detach the vesicles from the Pt wires. Subsequently, the GUVs were cooled to room temperature in a time span of approximately 5 h in an oven (J.P. Selecta, Barcelona, Spain) using a temperature ramp (~0.2 °C/min). This step was done in order to achieve equilibrium conditions in our samples. Once the solution reached room temperature, the vesicles were transferred to an

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