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Psychosine remodels model lipid membranes at neutral pH

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ABSTRACT

β-Galactosylsphingosine or psychosine (PSY) is a single chain sphingolipid with a cationic group, which is degraded in the lysosome lumen by β-galactosylceramidase during sphingolipid biosynthesis. A deficiency of this enzyme activity results in Krabbe's disease and PSY accumulation. This favors its escape to extralysosomal spaces, with its pH changing from acidic to neutral. We studied the interaction of PSY with model lipid membranes in neutral conditions, using phospholipid vesicles and monolayers as classical model systems, as well as a complex lipid mixture that mimics the lipid composition of myelin. At pH 7.4, when PSY is mainly neutral, it showed high surface activity, self-organizing into large structures, probably lamellar in nature, with a CMC of 38 ± 3 μM. When integrated into phospholipid membranes, PSY showed preferential partition into disordered phases, shifting phase equilibrium. The presence of PSY reduces the compactness of the membrane, making it more easily compressible. It also induces lipid domain disruption in vesicles composed of the main myelin lipids. The surface electrostatics of lipid membranes was altered by PSY in a complex manner. A shift to positive zeta potential values evidenced its presence in the vesicles. Furthermore, the increase of surface potential and surface water structuring observed may be a consequence of its location at the interface of the positively charged layer. As Krabbe's disease is a demyelinating process, PSY alteration of the membrane phase state, lateral lipid distribution and surface electrostatics appears important to the understanding of myelin destabilization at the supramolecular level.

1. Introduction

Psychosine or β-galactosylsphingosine (PSY) (Scheme 1) is an intermediate in the biosynthesis of sphingolipids, occurring in the lysosome lumen. It is degraded by the enzyme β-galactosylceramidase. A deficiency of this enzyme activity results in the progressive accumulation of PSY, 10- to 100-fold above its normal concentration [1], being responsible for so-called globoid cell leukodystrophy or Krabbe's disease. This is an inherited autosomal disorder that leads to demyelination, infiltration of macrophages into the brain parenchyma and early death [2]. PSY is a highly cytotoxic glycolipid in the 20–50 μM range [3]. Its mechanism of toxicity appears to occur through pleiotropic factors, including dysfunctions in several metabolic pathways [4]. There is evidence that this amphiphilic molecule exerts its pathological effect by partitioning into membranes and affecting their function [1,3,5,6]. It is also known that PSY is hemolytic [4] and induces

Abbreviations: (PSY), psychosine or β-galactosylsphingosine; (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; (DMPC), 1,2-dimiristoyl-sn-glycero-3-phosphocholine; (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; (DOPS), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt); (bSM), brain sphingomyelin; (CHO), cholesterol; (SULF), sulfatide; (Gal-Cer), galactosyl ceramide; (GANG), total gangliosides; (SPH), sphingosine; (PE-Rho), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl); (laurdan), 6-dodecanoyl-2-dimethyl-aminopnaphthalene; (GP), Laurdan generalized polarization; (DPH), 1,6-dipheniyl-1,35,-hexatriene; (r), fluorescence anisotropy; (MM), lipid mixture mimicking myelin; (π), surface pressure; (Cs^{-1}), compressibility modulus; (BAM), Brewster angle microscopy; (MLVs), multilamellar vesicles; (LUVs), large unilamellar vesicles; (GUVs), giant unilamellar vesicles; (CMC), critical micellar concentration; (LE), liquid-expanded phase; (LC), liquid-condensed phase; (LO), liquid-ordered phase; (LD), liquid-disordered phase

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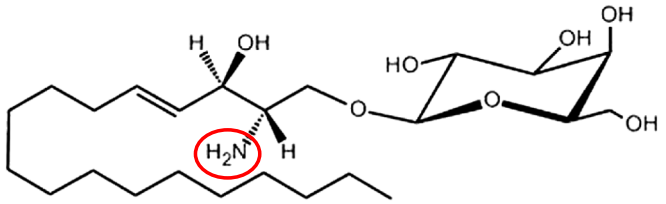

Scheme 1. Chemical structure of β-galactosylsphingosine or psychosine (PSY). The ionizable amine group with a pKa of 7.18 is highlighted.

permeabilization of liposomes [3].

Typically, biomembranes contain 10–16 mol% of negatively charged lipids [7], meaning that cell membranes are anionic. Natural cationic lipids are very rare and are present in very low quantities in physiological conditions. As far as we know, they are circumscribed to a few single chain sphingolipids, such as sphingosine (SPH), sphinganine and their glycosylated derivatives, glycosylsphingosine and PSY [4,8]. SPH has an apparent pKa of 9.1, and thus is cationic in the pH range that concerns physiological environments. On the other hand, PSY show a pKa of 7.18 [9], indicating that, in its natural environment - the acidic lysosomal lumen, PSY is mainly charged. At pH 4.5, PSY behaves as a mild soluble amphiphile, organized as micelles of ~14 nm in diameter and a critical micellar concentration (CMC) of 1.26 mM [9]. However, in Krabbe's disease, PSY is present at high concentrations and escapes the lysosome lumen to accumulate in extralysosomal spaces, such as the cytoplasm or extracellular milieu, at pH close to neutrality (~7.4). At this pH, PSY is only partially charged, forming stable Langmuir films [10] and large structures in bulk, whilst its CMC drops to the micromolar range [9]. Its properties then differ from those of the fully charged lipid and a more lipid-like character may prevail.

This work explores the interaction of PSY with model lipid membranes in neutral pH conditions. We used phospholipid membranes in different phase states as classical model systems. Additionally, we explored PSY interaction with a complex lipid mixture of phospholipids, sphingolipids and cholesterol that mimics the lipid composition of myelin [11–13]. As Krabbe's disease is a demyelinating process, our results are relevant to the supramolecular interpretation of the mechanism of biomembrane perturbation by PSY.

2. Materials and methods

2.1. Chemicals and reagents

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dimiristoyl-sn-glycero-3-phosphocholine (DMPC). 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt) (DOPS), brain sphingomyelin (bSM), cholesterol (CHO) and 1-β-galactosyl-sphing-4-enine (psychosine or PSY) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (PE-Rho) were purchased from Avanti Polar Lipids, Inc. (Alabama, U.S.A.). Sulfatide (SULF), galactosyl ceramide (Gal-Cer) and total gangliosides (GANG) were purified from bovine brain as reported in Ref. [10] and were all generously provided by Dr. Bruno Maggio. 1,6-Dipheniyl-1,35,-hexatriene (DPH) and 6-dodecanoyl-2-dimethyl-aminopnaphthalene (laurdan) were obtained from Invitrogen (Eugene, Oregon, USA). Chloroform, Methanol, NaCl and Tris-Base were supplied by Merck (Darmstadt, Germany). Deionized water with a resistivity of 18 MΩ cm was obtained from a Milli-Q Gradient System (Millipore, Bedford, MA). The lipid mixture mimicking myelin (MM) was chosen to fit bibliographic data [11,13] as follows: CHO, 38 mol%; POPC, 7 mol%; DOPE, 19 mol%; DOPS, 6 mol%; bSM, 10 mol%; SULF, 5 mol%; Gal-Cer 15 and GANG 1 mol%.

2.2. Methods

2.2.1. Adsorption experiments

The adsorption of PSY into the air-water interface was performed by injection of increasing volumes of a stock solution PSY (30 mM) in ethanol into the aqueous subphase under continuous stirring. The subphase was Tris-HCl 10 mM NaCl 135 mM, pH 7.4. A miniature circular trough of 1 mL and 3.14 cm^2 was used for surface tension/pressure measurements coupled to a Langmuir film balance (Kibron MicroTrough, Helsinki, Finland). The change in surface pressure was registered as a function of time. The surface activity curves were fitted via non-linear least-squares regression analysis as follows:

$$\Gamma = \frac{C}{RT}\frac{d\pi}{dC}, \tag{1}$$

where Γ is the amphiphile surface excess concentration, R is the gas constant, T is the temperature, C is the subphase amphiphile concentration and π is the surface pressure defined as the difference between the surface tension of the bare air/buffer interface and the surface tension reached after equilibration with the amphiphile added to the subphase

$$(\pi = \gamma_0 - \gamma), \tag{2}$$

From the maximum Γ value, the area occupied by a single amphiphile molecule at maximal surface concentration can be calculated as:

$$A = {}^{1}\!/\!_{N\Gamma_{max}}, \tag{3}$$

where N is Avogadro's constant. All experiments were performed at 22 ± 1 °C.

2.2.2. Surface titration experiments

The PSY uptake capacity by lipid monolayers at 30 mN/m was tested by means of surface titration experiments [14]. We spread a known amount of lipids onto the air/saline solution surface (at constant area) up to 30 mN/m and then stepwise co-spread a chloroform solution of PSY, registering the resultant π increase after solvent evaporation. At a certain amount of PSY added, a saturating π value was reached, which did not increase with further addition of drug. We determined the PSY mole fraction at which the π reached 50% of the saturating value (X_{50}).

2.2.3. Surface pressure-area isotherms

Monomolecular lipid films were obtained by spreading adequate aliquots of chloroform solutions of lipids onto the aqueous surface of a Teflon™ trough incorporated in a KSV NIMA minitrough equipment (Biolin Scientific AB, Västra Frölunda Sweden). The surface pressure was determined with a Pt plate using the Wilhelmy method. After solvent evaporation and relaxation at π ≤ 0.5 mN/m (~5 min), the film was compressed isometrically at a rate of 6 ± 1 $Å^2 \cdot molec^{-1} \cdot min^{-1}$ until reaching the collapse pressure by reducing the area between two Delrin™ barriers. Their lateral movement over the trough surface was controlled and registered by an electronic unit. The mean molecular area (MMA) was taken as the total monolayer area divided by the total number of molecules placed at the interface. The absence of surface-active impurities in the subphase solutions or in the spreading solvents was checked routinely. Surface elasticity upon compression was assessed by means of the compressibility modulus (Cs^{-1}), which was calculated from the isotherm data as [15]:

$$C_s^{-1} = -MMA\left(\frac{\delta\pi}{\delta MMA}\right)_T \tag{4}$$

The surface potential of the film was simultaneously measured with a surface ionizing electrode formed by a ^{241}Am plate positioned ~5 mm above the monolayer surface, and a reference $Ag/AgCl_2$ (3 M) electrode connected to the aqueous subphase.