BBA - Biomembranes 1866 (2024) 184271

Contents lists available at ScienceDirect

BBA - Biomembranes

journal homepage: www.elsevier.com/locate/bbamem

Evidence for Ca^{2+}-induced structural change in diluted GD3 ganglioside dispersions

Julia B. Ejarque [a], Evandro L. Duarte [b], M. Teresa Lamy [b], Julio H.K. Rozenfeld [a,*]

[a] Departamento de Biofísica, Escola Paulista de Medicina, Universidade Federal de São Paulo, R. Botucatu 862, 04023-062 São Paulo, SP, Brazil
[b] Instituto de Física, Universidade de São Paulo, Rua do Matão 1371, 05508-090 São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
GD3 ganglioside
Calcium
Micelles
Vesicles
Electron paramagnetic resonance spectroscopy

GD3 is a ganglioside involved in cancer malignancy [1,2] and neurodegenerative disorders [3]. It has two negatively charged sialic acid residues (Fig. 1A), which could interact with Ca^{2+}, a cation that is an important regulator of the structure and function of gangliosides [4,5].

GD3 from bovine milk (Lot # 860060-01-010) was obtained from Avanti Polar Lipids. GD3 was solubilized in mixtures of chloroform/methanol (2:1 *v*/v) and dried under a nitrogen stream. For EPR experiments, 0.8 mol% of 5-PCSL or 0.3 mol% of 16-PCSL were added to the solvent mixtures. The lipid films were dispersed in HEPES buffer (10 mM, pH 7.4) by heating for 20 min at 87 °C in a water bath. Heating was accompanied by vortexing at every 5 min. For Ca^{2+} assays, the buffer solution was prepared with a final $CaCl_2$ concentration of 100 mM by mixing 40 mL of a 20 mM HEPES solution with 32 mL of a 250 mM $CaCl_2$ solution, and adding ultrapure water to a final volume of 80 mL.

Differential Scanning Calorimetry (DSC) thermograms were obtained in a Microcal VP-DSC microcalorimeter (Microcal Inc., Northampton, MA, USA). An annealing (heating) scan of 90 °C/h was performed prior to all experiments. Scans were performed with at least two samples prepared in different days. Enthalpies of the thermal events (ΔH) were obtained by integrating the areas under the thermograms using the Microcal Origin software. ΔH values are shown with standard deviations (s.d.) for experiments with samples prepared in different dates.

Electron Paramagnetic Resonance (EPR) spectra in the X band were obtained in a Bruker EMX spectrometer equipped with a high sensitivity cavity (ER4119HS, Bruker). The microwave power was 13.4 mW, the modulation frequency was 100 kHz, and the modulation amplitude was 1 G. A Bruker BVT-2000 variable temperature device was used to keep sample temperatures within 0.1 °C. Empirical parameters such as the maximum hyperfine splittings (A_{max}) and the low (h_{+1}) and central (h_0) field line amplitudes were measured directly from spectra using the WinEPR software (Bruker). Each experiment was performed at least in duplicate and error values correspond to standard deviations.

DSC heating thermograms of diluted GD3 dispersions are shown in Fig. 1B. In absence of Ca^{2+}, a single broad and weak endothermic peak is observed around 20 °C. In presence of Ca^{2+}, a complex thermotropic behavior is observed: more intense endothermic peaks are observed at around 9 °C, 26 °C and 37 °C. The thermal events are more energetic in presence (ΔH = 2.7 kcal/mol ± 0.1 s.d.) than in absence of Ca^{2+} (ΔH = 0.3 kcal/mol ± 0.1 s.d.).

The thermotropic behavior of GD3 in absence of Ca^{2+}, with a small endothermic peak around 20 °C, is similar to the one observed for GD1a, a disialoganglioside that forms micelles [6,7]. In contrast, the complex thermotropic behavior observed in presence of Ca^{2+} recalls the one observed for GM3, the precursor of GD3 that spontaneously forms vesicles [8]. The complex GM3 behavior involves the rearrangement of both the hydrophobic chains and the hydrophilic headgroups [8].

The paramagnetic probes 5-PCSL and 16-PCSL (Fig. 1A) give structural information about the region close to the lipid-water interface and the deeper region of hydrophobic tails, respectively. EPR spectra of these probes in GD3 dispersions are shown in Fig. 1C. Without Ca^{2+}, the

* Corresponding author.
E-mail address: julio.rozenfeld@unifesp.br (J.H.K. Rozenfeld).

https://doi.org/10.1016/j.bbamem.2024.184271
Received 5 December 2023; Received in revised form 2 January 2024; Accepted 4 January 2024
Available online 8 January 2024

spectra of both probes show features of a relatively fluid environment in temperatures as low as 5 °C (Fig. 1C). These spectra are very similar to the ones observed for lipid micelles in a comparable temperature range [9].

For GD3 with Ca^{2+}, the spectra of both probes are typical of a more packed and/or organized micro-environment at all temperatures tested (Fig. 1C). These spectra resemble the ones of lipid vesicles [9] and, at temperatures below 30 °C, they are similar to those observed in lamellar gel phases [10].

The maximum hyperfine splitting (A_{max}), which can be directly measured in the 5-PCSL spectra (Fig. 1C), is an empirical parameter that gives information about the packing or viscosity of assemblies, because Amax values decrease as packing or viscosity decrease [11]. This is observed as temperature increases (Fig. 1D). The Amax values increase substantially in presence of Ca^{2+}, indicating that the region near the interface becomes more packed or viscous in presence of this cation (Fig. 1D).

Notably, the Amax values of 5-PCSL at 10 °C and 50 °C in GD3 without Ca^{2+} (Fig. 1D) are very similar to the ones observed in micelles at the same temperatures [9]. In contrast, the Amax values in presence of Ca^{2+} (Fig. 1D) are very similar to the ones observed for a DPPC bilayer at these temperatures [9].

Since Amax values cannot be accurately determined from the more isotropic 16-PCSL spectra (Fig. 1C), the ratio of the low and central field line amplitudes (h_{+1}/h_0) can be employed to access molecular packing, because h_{+1}/h_0 values increase as assemblies become less packed [11]. The h_{+1}/h_0 values are much smaller in presence of Ca^{2+}, showing that this ion rigidifies the core of the assemblies at all temperatures tested (Fig. 1D).

The DSC and EPR data suggest (Figs. 1B, C and D) that GD3 spontaneously forms micelles in a diluted regime, as observed for other disialogangliosides such as GD1a and GD1b [7]. This is a result of the

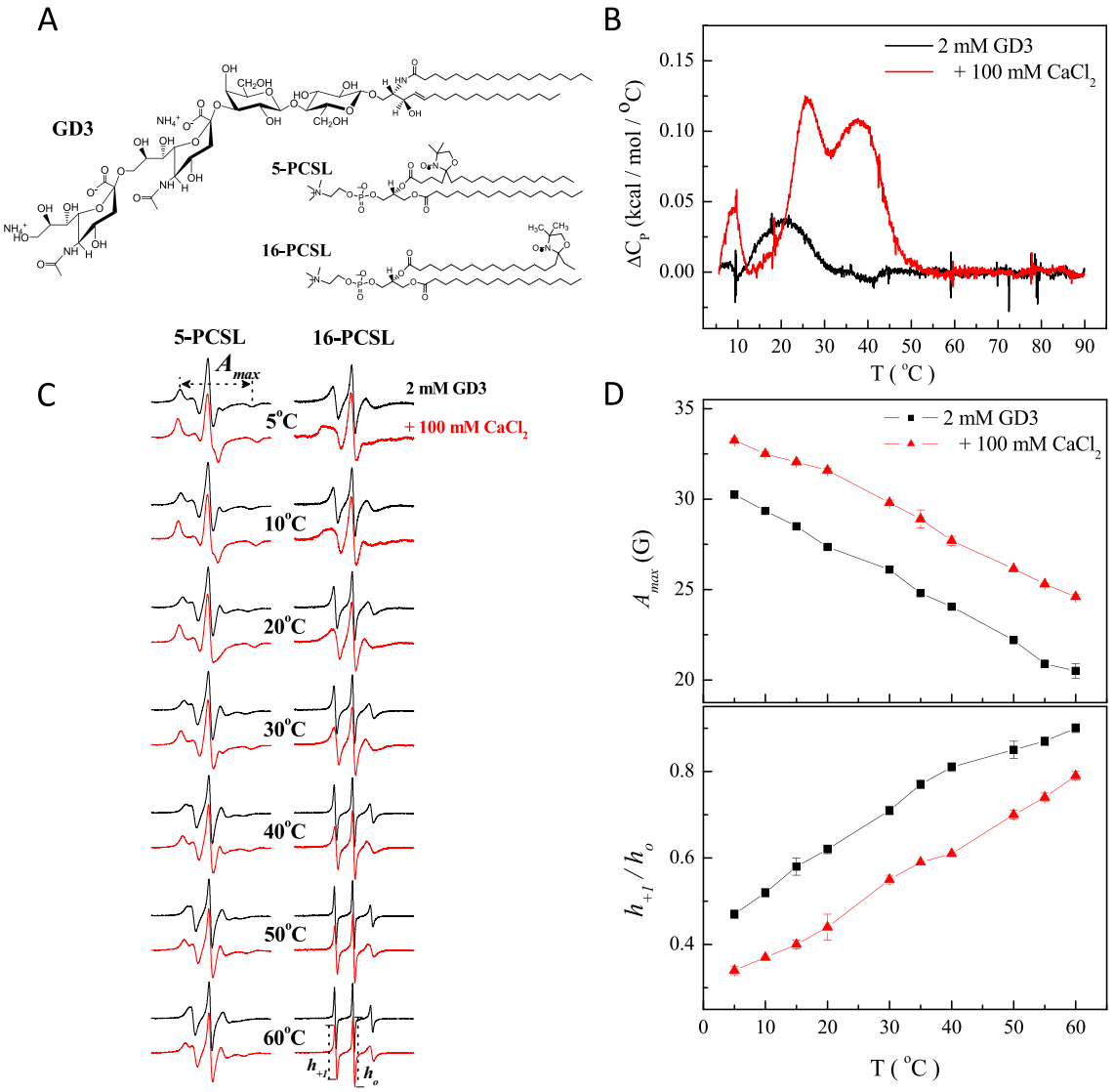

Fig. 1. Structure and thermotropic behavior of GD3 dispersions in absence or presence of Ca^{2+}. (a) Molecular structures of GD3 and spin labels 5-PCSL and 16-PCSL; (b) heating thermograms of 2 mM GD3 dispersions in absence (black line) and presence of 100 mM $CaCl_2$ (red line) at scan rate of 20 °C / h; (c) EPR spectra of 5- and 16-PCSL embedded in GD3 dispersions in absence (black) and presence of 100 mM $CaCl_2$ (red line). The maximum hyperfine splitting (Amax) of 5-PCSL spectra and the amplitudes of low (h_{+1}) and central (h_0) field lines of 16-PCSL spectra are indicated. The total spectra width is 100 G; (d) Amax and h_{+1}/h_0 values as a function of temperature for GD3 dispersions in absence (black squares) and presence (red triangles) of 100 mM $CaCl_2$. These empirical parameters were measured directly from the spectra of 5-PCSL and 16-PCSL, respectively, as signaled in (c). Error bars correspond to standard deviations of at least two independent experiments. All samples were prepared in 10 mM HEPES buffer pH 7.4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

strong contribution of their bulky and charged headgroups to the packing parameters [8]. Ca^{2+} induces changes in DSC and EPR data (Fig. 1B, C and D) that are consistent with the formation of GD3 lamellar structures. Ca^{2+} is able not only to shield the electrostatic repulsion of sialic acids, but also to change the polarity, the hydrogen bonding and the state of hydration of ganglioside headgroups [12]. These effects might facilitate a reorientation of the GD3 headgroups that results in the formation of a lamellar structure.

Vesicles spontaneously formed by GM3, the monosialoganglioside precursor of GD3, exhibit a reorientation of headgroups that facilitate molecular packing [8,13]. In this case, a solid-disordered phase is formed [8,13]. This phase is an intermediate between the usual gel and fluid phases, and consists of an ordered headgroup region and a disordered chain core [8,13]. This solid-disordered phase might explain the GM3 role in tumor progression by facilitating shape-dependent recognition [8].

The pathophysiological roles of GD3 depend on its ability to regulate cell death in a context-dependent manner [2,3,14]. This context could be provided by Ca^{2+}, which was shown to synergize with GD3 to cause mitochondrial damage and apoptosis [15]. The results shown here suggest that Ca^{2+} induces important structural changes in GD3 dispersions. These changes could be involved in the mechanisms of action of GD3, and deserve further investigation.

CRediT authorship contribution statement

Julia B. Ejarque: Investigation, Formal analysis. **Evandro L. Duarte:** Writing – review & editing, Project administration, Methodology, Formal analysis. **M. Teresa Lamy:** Writing – review & editing, Resources, Formal analysis. **Julio H.K. Rozenfeld:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julio H K Rozenfeld reports equipment, drugs, or supplies was provided by State of Sao Paulo Research Foundation. Julio H K Rozenfeld reports equipment, drugs, or supplies was provided by National Council for Scientific and Technological Development. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by grants #2016/19077-1, #2016/13368-4, and #2021/01593-1, São Paulo Research Foundation (FAPESP). J.B. E. was supported by a CNPq fellowship and M.T.L. is recipient of a CNPq research fellowship.

References

[1] S. Groux-DeGroote, Y. Guérardel, P. Delannoy, Gangliosides: structures, biosynthesis, analysis and roles in cancer, ChemBioChem 18 (2017) 1146–1154.
[2] X. Jin, G.-Y. Yang, Pathophysiological roles and applications of glycosphingolipids in the diagnosis and treatment of cancer diseases, Prog. Lipid Res. 91 (2023) 101241.
[3] S. Sipione, J. Monyror, D. Galleguillos, N. Steinberg, V. Kadam, Gangliosides in the brain: physiology, pathophysiology and therapeutic applications, Front. Neurosci. 14 (2020) 572965.
[4] Y. Xu, et al., Gangliosides play important roles in the nervous system by regulating ion concentrations, Neurochem. Res. 47 (2022) 1791–1798.
[5] M. Cebecauer, et al., Membrane lipid nanodomains, Chem. Rev. 118 (2018) 11259–11297.
[6] B. Maggio, T. Ariga, J.M. Sturtevant, R.K. Yu, Thermotropic behavior of glycosphingolipids in aqueous dispersions, Biochemistry 24 (1985) 1084–1092.
[7] S. Sonnino, A. Prinetti, L. Mauri, V. Chigorno, G. Tettamanti, Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains, Chem. Rev. 106 (2006) 2111–2125.
[8] L. Cantu', E. Del Favero, P. Brocca, M. Corti, Multilevel structuring ofganglioside-containing aggregates: from simple micelles to complex biomimetic membranes, Adv. Colloid Interf. Sci. 205 (2014) 177–186.
[9] R. Bartucci, S. Belsito, L. Sportelli, Spin-label electron spin resonance studies of micellar dispersions of PEGs-PEs polymer-lipids, Chem. Phys. Lipids 124 (2003) 111–122.
[10] L.S. Martins, E.L. Duarte, M.T. Lamy, J.H.K. Rozenfeld, DODAB vesicles containing lysophosphatidylcholines: the relevance of acyl chain saturation on the membrane structure and thermal properties, Biophys. Chem. 300 (2023) 107075.
[11] J.H.K. Rozenfeld, E.L. Duarte, T.R. Oliveira, M.T. Lamy, Structural insights on biologically relevant cationic membranes by ESR spectroscopy, Biophys. Rev. 9 (2017) 633–647.
[12] P.D. Thomas, G.L. Brewer, Gangliosides and synaptic transmition, Biochim. Biophys. Acta 1031 (1990) 277–289.
[13] S. Motta, et al., Hierarchical ordering of sugar based amphiphiles, Mol. Cryst. Liq. Cryst. 500 (2009) 155–165.
[14] M. Sorice, et al., Ganglioside GD3 as a raft component in cell death regulation, Anti Cancer Agents Med. Chem. 12 (2012) 376–382.
[15] F. Malisan, R. Testi, GD3 ganglioside and apoptosis, Biochim. Biophys. Acta 1585 (2002) 179–187.