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Review

Imaging cerebroside-rich domains for phase and shape characterization in binary and ternary mixtures

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ABSTRACT

The objective of this paper is to review phase behavior and shape characterization of cerebroside-rich domains in binary and ternary lipid bilayers, as obtained by microscopy techniques. These lipid mixtures provide a format to examine molecular (e.g. headgroup, tail unsaturation, and tail hydroxylation) and thermodynamic (e.g. temperature and mole percentages) factors that determine phase behavior, molecular partitioning, crystal/atomic scale structure, and microstructure/shape (particularly of phase-separated domains). Microscopy can provide excellent spatial (often with high resolution) characterization of cerebroside-rich domains (and their surroundings) to identify, describe or infer with high certainty these characteristics. In the introduction to this review we review briefly the molecular structure, phase behavior, and intermolecular interactions of cerebrosides, in comparison to ceramides and sphingomyelins and in some binary and biological systems. The bulk of the review is then devoted to microscopy investigations of cerebroside-rich domain microstructure and shape dynamics in binary and ternary (one component is cholesterol) systems. Quantitative and/or high-resolution microscopy techniques have been used to interrogate cerebroside-rich domains such as freeze-fracture electron microscopy, atomic force microscopy, imaging elipsometry, two-photon fluorescence microscopy, and LAURDAN generalized polarization in addition to the laboratory workhorse technique of epifluorescence microscopy that allows a quick often qualitative assessment of microstructure and dynamics. We particularly focus on the information these microscopy investigations have revealed with respect to phase behavior, cholesterol partitioning, domain shape, and determinants of domain shape.

Contents

1. Introduction

Cerebrosides are double-tailed ceramide (Cer) lipids bound in glycosidic linkage through the primary hydroxyl to either of two monosaccharides (galactose or glucose). This review will include only membrane domains rich in the former, galactocerebrosides, commonly referred to as simply cerebrosides or galactosylceramide (GalCer) lipids. The latter are referred to as glucocerebrosides or glucosylceramide (GlcCer) lipids. The presence of a 2-hydroxy acyl chain is found in approximately 40–60 mol% of naturally occurring cerebrosides [1]. In comparison to cerebrosides, sphingomyelins have a phosphorylcholine group bound to the ceramide lipid. Cerebrosides,

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ceramides, and sphingomyelins are all found to be highly saturated in natural sources. These are all referred to as sphingolipids because of the presence of the sphingosine base (an 18-carbon amino alcohol with a carbon double bond adjacent to the secondary hydroxyl).

Cerebrosides make up approximately 20% of the lipid of the myelin sheath of nerve tissue [2,3], which acts as a multilamellar insulator around axons, allowing fast conduction of electrical impulses. Effective conduction of nerve impulses is quite specific to the presence of GalCer; replacement in knockout mice by GlcCer results in conduction similar to unmyelinated axons [4]. Cerebrosides are also found in significant quantities in the epithelial cells of the small intestine and colon [5] and in the granular layer of the epidermis of skin [6]. Cerebrosides have been studied for their anti-HIV properties. Indeed, it has known since the mid 1990s that the HIV envelope glycoprotein (Env) binds to GalCer [7]. This interaction is fairly specific to galactose demonstrated by the inactivity of GlcCer in binding HIV Env [8].

For cerebrosides, like other monoglycosylceramides, the order–disorder transition temperature T_m is essentially independent of acyl chain length [9]. However the presence of a 2-OH group in the acyl chain lowers the T_m. For example, 18:0-nonhydroxy fatty acid (NFA)-GalCer and 18:0-2-hydroxy fatty acid (HFA)-GalCer exhibit T_m at 83 °C and 70 °C, respectively [9]. Cerebrosides extracted from Bovine brain tissue (bb cerebrosides) exhibit a broad transition with a T_m of 67 °C [10,11] reflecting primarily a mixture of 2-hydroxylated (~60%) and non-hydroxylated GalCers, but also to a smaller degree a mixture of different chain lengths and saturated and unsaturated lipids. Any unsaturated lipids are primarily mono-unsaturated [12]. In addition, it appears that all monoglycosylceramides exhibit metastable gel states mediated by inter-lipid hydrogen bonding [13]. Curaloto [14] suggested that a function of the 2-OH group in brain cerebrosides may be to eliminate one of these metastable states where dehydration in cerebroside-rich membranes occurs close to body temperature [13]. Cerebrosides and other monoglycosylceramides have exceptionally high T_m values as a result of the extensive hydrogen bonding capability of the saccharide headgroup [9,15]. The crystal structure of a synthetic GalCer molecule (in the presence of ethanol) displayed a bilayer arrangement, with the plane of the sugar ring almost parallel to the bilayer plane [15]. The lateral hydrocarbon chain packing was of a hybrid type (HS2). Maggio and coworkers [16] have recently provided a discussion of the biophysical properties of glycosphingolipids.

In comparison, sphingomyelins contain a ceramide backbone, similar to cerebrosides, but with a phosphorylcholine group replacing the saccharide group. Sphingomyelins have an amide linkage in the ceramide backbone, which is capable of acting as a hydrogen bond donor [9]. Similar to cerebrosides, synthetic sphingomyelins with a single acyl chain type exhibit phase metastability [17,18], and a dependence of T_m on acyl chain length, which is significantly weaker than that of the phosphatidylcholines (PCs) [19]. However, sphingomyelins have much lower T_m values compared to single-chain length cerebrosides e.g., 85 °C for N-palmitoyl-GalCer [20] compared to 41 °C for N-palmitoyl-sphingomyelin [21,22]. Similar to cerebrosides, self-assembling ceramide structures have a high T_m due to strong intermolecular interactions (mainly hydrogen bonds) promoted by the small headgroup allowing close interaction of the ceramide headgroups. For example, 16:0-NFA-ceramide exhibits a broad exothermic transition at approximately 50–70 °C [23].

Studies of binary mixtures containing cerebrosides are limited. Curoloto [24] used DSC to develop a phase diagram for hydrated bilayers of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and bb cerebrosides. This system displayed monotectic behavior similar to other binary systems with widely varying T_m values (e.g. dilauryl phosphatidylcholine (DLPC)/distearoyl phosphatidylcholine (DSPC)) [25], i.e. upon cooling the bilayer transitioned from one liquid-disordered phase to coexistence of a liquid-disordered and solid-ordered like phase present at most POPC/bb cerebrosides ratios and most physiologically relevant temperatures. The flatness of the solidus transition indicates that POPC and bb cerebrosides are nearly completely immiscible in the gel state similar to DLPC/DSPC. Similarly, Magio et al. [26] found that bb cerebrosides were immiscible with DPPC in the gel state at bb cerebrosides contents up to 46 mol%. Interestingly, the phase diagram resembles a eutectic where (from ~0 to ~46%) one well-mixed liquid-disordered phase exists, and upon cooling transitions directly to two solid-ordered phases at approximately 41 °C. Ruocco and Shipley [27] used DSC and X-ray diffraction to derive a phase diagram for hydrated mixtures of 16:0-NFA-GalCer and cholesterol. Upon cooling below ~40 °C, the mixture transitioned from a glycolipid/cholesterol liquid crystalline phase (presumably a liquid-ordered phase) to a state of immiscibility of 16:0-NFA-GalCer and cholesterol. However, it must be recognized that 16:0-NFA-GalCer has a higher T_m than an extract such as bb cerebrosides; if 16:0-NFA-GalCer were replaced by bb cerebrosides this ~40 °C transition temperature might be decreased significantly.

Several studies have scrutinized the ability of cerebrosides to promote liquid-ordered domain formation in ternary systems e.g. [27–30]. Comparison between these studies can be difficult because of differences in lipids, component ratios, characterization methods, and sample preparation methods (such uncertainties are reviewed in [31]). However, the general consensus appears to be that sphingomyelin promotes liquid-ordered domain formation more effectively than monoglycosylsaccharides, including cerebrosides. At the same time, a number of studies on model systems have indicated that both sphingolipid structure and co-lipid characteristics are important determinants in interaction preferences of cholesterol [32]. Recent studies indicate that glycosphingolipids in biological membranes participate in cholesterol-dependent and cholesterol-independent "raft" and domain formation; in particular cholesterol-independent glycosynapses (glycosignalling domains) participate in cell–cell interaction and recognition [33–36]. In addition, in B16 melanoma cells, similar amount of detergent-resistant membrane (usually assumed to be liquid-ordered) was isolated from B16 melanoma cells with a normal sphingomyelin/glycosphingolipid ratio or in which glycosphingolipid has been replaced (through mutation) by sphingomyelin [37] inferring that cholesterol can interact as favorably with glycosphingolipids as sphingomyelins.

In addition to the studies discussed above, several microscopy studies have been performed on binary and ternary mixtures containing cerebrosides. We will cover these microscopy studies [1,38–44] here. The microscopy work is generally fairly recent and addresses questions with respect to cerebroside phase behavior, cholesterol preference, and domain microstructure. In addition, the work addresses broader issues regarding intermolecular interactions in lipid mixtures and the impact of thermodynamic and diffusional processes in domain shape. The techniques used in these studies are primarily epifluorescence microscopy, freeze fracture microscopy, atomic force microscopy, imaging elipsometry, two-photon fluorescence microscopy ,and LAURDAN generalized polarization.

2. Binary mixtures

2.1. Domain phase and shape

Phase separation in PC/cerebrosides lipid bilayers has been investigated using imaging techniques. Blanchette et al. [38] formed giant unilamellar vesicles (GUVs) of DLPC/bb cerebrosides (1/1 and 3/1 mol/mol), at room temperature, that included 1 mol% of the fluorescent dye NBD-PC. Micron-scale dark domains on each GUV were generally observed by fluorescence microscopy. These domains were leaf-like in shape (Fig. 1, 0% chol), rotated as rigid bodies and did not coalesce upon contact or become significantly rounded over viewing times of approximately 1 hour. Leaf-shaped domains with