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Ceramide effects in the bicelle structure

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Abstract

This work describes the effects of the inclusion of ceramides with different chain lengths in the structure of bicelles formed by dimyristoylphosphocholine (DMPC) and dihexanoylphosphocholine (DHPC). By using a number of physical techniques we observed that the bicellar structures were affected by both the concentration and the type of ceramide. The ^{31}P nuclear magnetic resonance showed that inclusion of the short chain ceramide in the system slightly affects the phosphorus resonance whereas the inclusion of the long chain ceramide promotes the differentiation of the peaks. The decrease in the ceramide chain length is associated with a less miscibility in DMPC. The values of *d*-spacing obtained by small angle X-ray scattering suggest that ceramides are organized in domains along the DMPC bilayer. The bicellar system studied supported the inclusion of 10 mol% of the long chain ceramide. This inclusion increased the particle size but did not affect the integrity of the structures. Freeze-fracture electron microscopy and dynamic light scattering techniques showed small bicelles with diameters around 15–20 nm and a low polydispersity index for this system. The inclusion of 10 mol% of the short chain ceramide in the bicellar system resulted in the formation of aggregates with two different morphologies: small rounded structures 15–20 nm in diameter and elongated structures 40 nm in length. The inclusion of 20 mol% of either of these two ceramides resulted in the formation of bigger structures in the range of 60–100 nm together with small bicelles.

Keywords: Bicelles; Ceramide domains; Small angle X-ray scattering; Freeze-fracture electron microscopy; ^{31}P nuclear magnetic resonance; Dynamic light scattering

1. Introduction

Bicelles are discoidal aggregates constituted by a flat dimyristoylphosphocholine (DMPC) bilayer, stabilized by a rim of dihexanoylphosphocholine (DHPC) in water [1]. These systems are normally used to orient membrane proteins that can be inserted in their structures [2,3], and to study the superficial interaction between proteins and phospholipid bilayers [4]. Given the usefulness of these structures as membrane models [5], the inclusion of specific cell membrane lipids in the bicelles is a suitable "in vitro" strategy to study the role played by these specific compounds.

Ceramide (*N*-acylsphingosine) is the dominant lipid component of epidermal stratum corneum. Moreover, this lipid is known to be involved in the regulation of several cellular responses to extracellular stimulus [6]. Crucial biological processes such as the cell proliferation [7], differentiation [8] and apoptosis [9] are induced by the increase in ceramide concentration in the cells, which activates various cellular mediators and reactions. The differences in ceramide structure are usually related to changes in the membrane physical properties and in the physiological state of the cells [10,11]. Given the importance of ceramides in cell functions and the growing interest of bicelles as a membrane model, the study of the effect of this lipid in the bicellar systems would be useful in clarifying events involving ceramide and phospholipids at cell membrane level.

We previously reported the self-assembly properties of various amphiphiles, the structural transitions involved in the

Abbreviations: C_{24}-Cer, bovine brain ceramide (type III); C_{14}-Cer, *N*-myristoyl-D-erythro-sphingosine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; cL, total lipid concentration; *q*, long/short chain phospholipid molar ratio; SAXS, small angle X-ray scattering; FFEM, freeze-fracture electron microscopy; ^{31}P NMR, ^{31}P nuclear magnetic resonance; DLS, dynamic light scattering; HD, hydrodynamic diameter; PI, polydispersity index.

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interactions of surfactants with liposomes [12], and the influence of ceramide in these interactions [13–15]. These works demonstrate the important role of ceramides in the stability and permeability of bilayers. The present study describes the effect of the incorporation of ceramides with different chain lengths into the DMPC/DHPC bicellar systems. To this end, as in our earlier works [16,17], we used small angle X-ray scattering (SAXS), dynamic light scattering (DLS), and freeze-fracture electron microscopy (FFEM) techniques to perform our studies. In addition, the ^{31}P NMR technique, which has been used by other authors to investigate bicellar systems [18–23], was also applied.

2. Materials and methods

2.1. Chemicals

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) and *N*-myristoyl-D-erythro-sphingosine (C_{14}-Cer) were purchased from Avanti Polar Lipids (Alabaster, Al). Deuterium oxide and bovine brain ceramide, type III (C_{24}-Cer) were acquired from Sigma (St. Louis, MO). According to the information supplied by the manufacturers, bovine brain ceramide (type III) contains primarily stearic (18:0) (31.6%, w/w) and nervonic (24:0) (48%, w/w) fatty acids over 99% of purity. The *N*-myristoyl-D-erythro-sphingosine is a synthetic product from D-erythro-sphingosine over 99% of purity. The molecular structures of both ceramides are displayed in Fig. 1.

2.2. Samples preparation

The following systems were prepared: (a) pure DMPC/DHPC, $q = 2$ (q = molar ratio DMPC/DHPC), (b) DMPC/DHPC, $q = 2$ including 10 mol% of C_{14}-Cer, (c) DMPC/DHPC, $q = 2$ including 10 mol% of C_{24}-Cer, (d) DMPC/DHPC, $q = 2$ including 20 mol% of C_{14}-Cer and (e) DMPC/DHPC, $q = 2$ including 20 mol% of C_{24}-Cer. The concentration of ceramide is given with respect to the long chain phospholipid concentration. Appropriate amounts of DMPC, DHPC and ceramide were mixed in a chloroform solution and evaporated to dryness using a rotary evaporator. The systems were hydrated to reach 20% (w/v) of total lipid concentration (cL) and then subjected to several cycles of sonication, freezing and heating until the samples became transparent. The measurements were carried out at room temperature using freshly made samples.

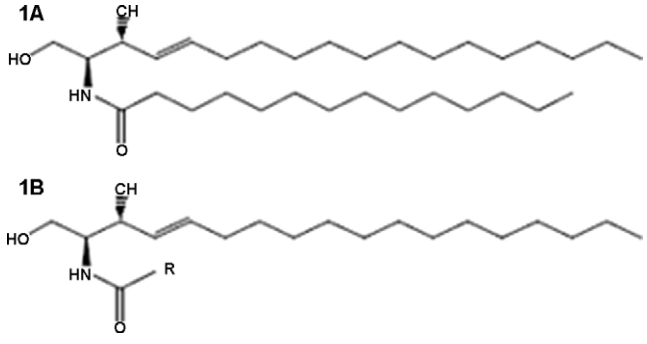

Fig. 1. Molecular structure of the two ceramides used. (A) The *N*-myristoyl-D-erythro-sphingosine, C_{14}-Cer and (B) the bovine brain ceramide type III, C_{24}-Cer. R = mixture of fatty acids, primarily stearic acid (18:0) (31.6%, w/w) and nervonic (24:0) (48%, w/w).

2.3. Dynamic light scattering

The hydrodynamic diameter (HD) and polydispersity index (PI) of the different systems were determined by means of a DLS equipment using a photocorrelator spectrometer (Malvern Autosizer 4700c PS/MV) equipped with an Ar laser source (wavelength 488 nm). Quartz cuvettes were filled with the samples, and all the experiments were thermostatically controlled. All the experiments were performed at 90° scattering angle. The analysis of the data obtained was performed using the version of the program CONTIN provided by Malvern Instruments, England.

2.4. Freeze-fracture electron microscopy

Freeze-fracture electron microscopy (FFEM) study was carried out according to the procedure described by Egelhaaf et al. [24]. About 1 μl of suspension was sandwiched between two copper platelets using 400-mesh gold grids as spacer. Then the samples were frozen by plunging in propane at −180 °C and fractured at −150 °C and 2×10^{-7} mbar in a Balzers BAF 300 freeze-fracturing apparatus (BAL-TEC, Liechtenstein). The replicas were obtained by unidirectional shadowing with 2 nm of Pt/C and 20 nm of C, and they were floated on distilled water and examined in a JEOL 1010 TEM electron microscopy at 80 Kv.

2.5. Small angle X-ray scattering (SAXS) measurements

SAXS measurements were carried out using a Kratky camera of small angle (M Braun) coupled to a Siemens KF 760 (3 kW) generator. Nickel-filtered radiation with wavelength corresponding to the Cu Kα line (1.542 Å) was used. The linear detector was PSD-OED 50 M-Braun, and the temperature controller was a Peltier KPR AP PAAR model. The sample was inserted between two Mylar sheets with a 1 mm separation. The SAXS curves were smoothed by fitting a polynomial of degree three to an increasing number of points as the channel number increased. The fitting software was designed to guarantee constant slopes and fixed peak position. The system uses a line collimated beam; therefore, to preserve sharpness, the smoothed curves were desmeared using the procedure of Singh et al. [25]. The SAXS curves are shown as a function of the scattering vector modulus

$$q = \frac{4\pi}{\lambda} \sin\theta \quad (1)$$

where θ is the scattering angle and λ the wavelength of the radiation (1.542 Å). The position of the diffraction peaks are directly related to the repeat distance of the molecular structure,