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Interactions of dipalmitoylphosphatidylcholine with ceramide-based mixtures

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ABSTRACT

The outermost layer of the skin, the stratum corneum (SC), acts as the natural physical barrier. The SC consists of corneocytes embedded in a crystalline lipid matrix consisting of ceramides, free fatty acids and cholesterol.

Although phospholipids are frequently present in topical formulations, no detailed information is reported on the interactions between phospholipids and SC lipids. The aim of this study was to examine the interactions between a model phospholipid, dipalmitoylphosphatidylcholine (DPPC) and synthetic ceramide-based mixtures (referred to as SC lipids).

(Perdeuterated) DPPC was mixed with SC lipids and the lipid organization and mixing properties were examined. The studies revealed that DPPC participates in the same lattice as SC lipids thereby enhancing a hexagonal packing. Even at a high DPPC level, no phase separated pure DPPC was observed.

When a DPPC containing formulation is applied to the skin surface it must partition into the SC lipid matrix prior to any mixing with the SC lipids. To mimic this, DPPC was applied on top of a SC lipid membrane. DPPC applied in a liquid crystalline state was able to mix with the SC lipids and participated in the same lattice as the SC lipids. However, when DPPC was applied in a rippled gel-state very limited partitioning of DPPC into the SC lipid matrix occurred. Thus, when applied to the skin, liquid crystalline DPPC will have very different interactions with SC lipids than DPPC in a (rippled-)gel phase.

1. Introduction

Several therapeutic and cosmetic formulations for topical skin health applications on the market contain phospholipids, most commonly phosphatidylcholine (lecithin). In general liquid crystalline phosphatidylcholine is utilized to form liposomes although phospholipids are also used as natural emulsifiers [1]. Very little information has been reported describing the interaction between phospholipids (particularly conformationally ordered phosphatidylcholine) and skin barrier lipids. In this work the interaction between dipalmitoyl phosphatidyl choline (DPPC) and two skin barrier lipid models was examined using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction. DPPC was selected as it is one of the most frequently used phospholipids and exists in an ordered gel phase at skin physiological temperatures (~32 °C).

The skin is composed of several morphologically distinct layers. The upper layer of the skin is the stratum corneum (SC). Human SC is a 10–15 μm thick layer consisting of corneocytes in a lipid matrix, which is responsible for the primary skin barrier function. The corneocytes are flat hexagonal-shaped dead cells, covalently linked together by corneodesmosomes, and filled with keratin filaments, bound water and hygroscopic molecules known as natural moisturising factor [2]. The free volume between corneocytes is filled with three major classes of lipids; free fatty acids (FFAs), ceramides (CERs) and cholesterol (CHOL) [3]. As the extra-cellular lipid domains form a continuous structure in the SC, molecules diffusing across the SC always have to pass through the lipid regions. For this reason, the SC lipids play an important role in skin barrier function and the arrangement of the lipids in lamellar domains is a key process in the formation of the skin barrier. X-ray diffraction studies of human SC have shown that the lipids are arranged in

Abbreviations: SC, stratum corneum; CER, ceramide; CHOL, cholesterol; FFA, free fatty acid; FTIR, Fourier transformed infrared spectroscopy; SAXD, small angle X-ray diffraction; FA24, lignoceric acid; DPPC, dipalmytoyl phosphatidyl choline; EOS, ester linked omega-hydroxy acyl chain linked to a sphingosine chain; NS, a non-hydroxy acyl chain linked to a sphingosine base; SPP, short periodicity phase; LPP, long periodicity phase

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two crystalline lamellar phases with repeat distances of 6 (short periodicity phase, SPP) and 13 nm (long periodicity phase, LPP), respectively [4]. The 2-dimensional crystalline packing of the lipid methylene groups is mainly orthorhombic, although a fraction of lipids also exhibits a hexagonal packing. The SC lipid domains are also very important when studying and interpreting the interaction of exogenous compounds with the SC. Several studies have reported on a range of interactions between chemical penetration enhancers and a range of emollient molecules with SC lipids [5–7].

While the SC is comprised of only three major lipid classes, the composition is very complex at the sub-class and species level. To date approximately 17 CER subclasses and 3 FFA subclasses have been identified in human SC [8–11]. Furthermore, each of these subclasses has a wide variation in chain length distribution. Consequently there are more than 1000 chemically distinct lipids in the SC. Recently we observed that a less complex well-defined lipid composition consisting of a limited number of CER subclasses, CHOL and the most abundant FFA assembles in both the LPP and the SPP [12] while adopting a predominantly orthorhombic packing at room temperature [12,13]. This has also been encountered in many other mixtures prepared with CERs, in which the mixing properties were strongly affected by lipid chain length distribution [14–16].

In this work, three or four component mixtures were selected that are able to form either the SPP or the LPP, respectively. For these mixtures we used synthetic ceramides. To determine the interactions between DPPC and SC lipids, the lipids were mixed with gradually increasing level of (perdeuterated) DPPC. We observed that high levels of DPPC are able to mix with the SC lipids and appear to participate in the same lattice as the SC lipids. To examine the partitioning of DPPC in a preformed SC lipid layer, a DPPC layer was placed on top of a SC lipid membrane and the partitioning and mixing of DPPC with the SC lipids was examined as a function of the DPPC phase behaviour (rippled gel phase or liquid crystalline (Lα)phase) [17]. These studies show that DPPC in liquid crystalline phase can partition into the SC lipid membrane and “mix” with the SC lipids.

2. Experimental section

2.1. Materials

Two synthetic CERs were used in our studies: 1) *N*-(omega-linoleyloxyacyl) sphingosine (abbreviation CER EOS) with an acyl chain of 30 carbon atoms and a sphingosine of 18 carbons, 2) a non-hydroxy acyl chain sphingosine (abbreviation CER NS). The acyl chain is 24 carbons and the sphingosine 18 carbons. For the molecular architecture of the CERs, see Supplement S1. The CERs were kindly provided by Evonik (Essen, Germany). Lignoceric acid (FA24) and CHOL were obtained from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). DPPC and perdeuterated DPPC was purchased from Avanti Polar Lipids (Alabaster, AL). Nuclepore polycarbonate filter disks with pore size of 50 nm were obtained from Whatman (Kent, UK). All solvents were of analytical grade and supplied by Labscan (Dublin, Ireland). The water used was of Millipore quality produced by Milli-Q water filtration system.

2.2. Preparation of the model lipid mixtures

The SC lipid mixtures were prepared using equimolar ratios of CER:CHOL:FA24. The CER consisted either of two CER subclasses, CER EOS and CER NS in a molar ratio of 40:60 (referred to as CER EOS/NS) or a single CER subclass, CER NS. Mixtures were prepared with a gradual increased level of (perdeuterated) DPPC varying in a molar ratio between CER:CHOL:FA24:DPPC = 1:1:1:0 to 1:1:1:4.

In preparing the model lipid mixtures, the appropriate amount of individual lipids was dissolved in chloroform:methanol (2:1) at a final concentration of 5 mg/ml and sprayed under a stream of nitrogen on a AgBr window using a Linomat IV (Camag, Muttenz, Switzerland). The Linomat device makes use of a Hamilton syringe (100 μl) and mechanics to spray a programmable volume of sample solution on either nucleopore filter disks (X-ray diffraction) or a AgBr window (Fourier transform infrared spectroscopy, FTIR). With the y-axis arm, the linomat is capable of spraying lipids in a rectangular shape. The spraying flow rate is 5.0 μl/min under a stream of nitrogen gas at a movement speed of 1 cm/s. After spraying, the lipid samples were equilibrated for 10 min at 85 °C. Then the mixture was cooled in approximately 30 min. Subsequently, the lipid layer was covered with 25 μl of a 50 mM acetate buffer (prepared with D_2O for FTIR studies) at pH 5. After buffer application, the sample was kept at 37 °C overnight to obtain a full hydration [18].

2.3. Fourier transform infrared spectral measurements

All spectra were measured on a Varian 670-IR FTIR or a Biorad FTS4000 FTIR spectrometer (Cambridge Massachusetts) equipped with a broad-band mercury cadmium telluride detector. The detector was cooled by liquid nitrogen. The sample cell was closed by two AgBr windows. The sample was under continuous dry air purge starting 1 h before the data acquisition. The spectra were measured in transmission mode. Each measurement consisted of a co-addition of 256 scans in a time period of 4 min. The resolution was 1 cm^{-1}. The thermotropic response was examined by increasing the sample temperature at a heating rate of 0.25 °C/min resulting in a 1 °C temperature rise per recorded spectrum until a temperature of 90 °C was reached. The software used was Resolutions Pro 4.1.0. from Varian.

2.4. Small-angle x-ray diffraction measurements

Small-angle x-ray diffraction (SAXD) was used to obtain information about the lamellar organization (i.e., the repeat distance of a lamellar phase). The scattering intensity I (in arbitrary units) was measured as function of the scattering vector q (in reciprocal nm). The latter is defined as $q = (4\pi\sin\theta) / \lambda$, in which θ and λ are the scattering angle and wavelength, respectively. From the positions of a series of equidistant peaks (q_n), the periodicity (d) of a lamellar phase was calculated using the equation $d = 2n\pi / q_n$, with n being the order number of the diffraction peak. One-dimensional intensity profiles were obtained by transformation of the 2D SAXD detector pattern from Cartesian (x,y) to polar (ρ,θ) coordinates and subsequently integrating over θ. All measurements were performed at the European Synchrotron Radiation Facility (ESRF, Grenoble) using station BM26B [19]. The wavelength of the X-ray and the sample-to-detector distance were 0.1033 nm and 2.1 m, respectively. The diffraction data were collected on a PILATUS 1M detector (1043 × 981 pixels) and 172 μm spatial resolution. The calibration of this detector was performed using silver behenate and aluminium oxide. The lipid membrane was mounted parallel to the primary beam in a sample holder with mica windows. All the diffraction data were collected for about 5 min at 25 °C.

2.5. Dynamic measurements

In addition to the mixing of DPPC and SC lipids in the organic solvent and subsequently spraying this mixture onto the support, the ability of DPPC to penetrate into a SC lipid membrane was also assessed. To examine this, DPPC was sprayed on one AgBr window using the same method as described above. The CER EOS/NS:CHOL:FA24 equimolar mixture was sprayed on another AgBr window. The skin lipid mixture was equilibrated at elevated temperatures (85 °C) and cooled to room temperature. Subsequently both, the skin lipid mixture and the DPPC, were hydrated overnight at 37 °C using an acetate buffer (pH 5). The excess water was removed and the two windows were sealed in the FTIR sample cell. The temperature of the cell was increased to either 32 or 37 °C, after having reached this temperature, the membrane was kept