Experimental report

04/05/2022

Proposal: 9-13-826 **Council:** 10/2018

Title: Effect of ceramide loss on the lipid ordering within the long periodicity phase of the stratum corneum

Research area: Biology

This proposal is a new proposal

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Samples: Ceramide EOS, Ceramide NS,cholesterol,C24 FFA

Instrument	Requested days	Allocated days	From	To
D16	6	6	13/09/2019	19/09/2019

Abstract:

The lipid matrix of the stratum corneum (SC) in the skin contains cholesterol (CHOL), free fatty acids (FFAs) and ceramides (CERs), arranged as lamellar phases with d-spacings of ca. 5.4 nm (SPP) and ca. 12.5 nm (LPP). The LPP structure is unique to the SC and is critical to its barrier function. In skin diseases like atopic dermatitis and psoriasis, as well as in aged skin, the barrier function of the SC is compromised, and this is often associated with a change in the proportions of CER and FFA. In the studies proposed here (which build on our previous studies of healthy skin LPP models using D16), we aim to move to diseased skin LPP model systems (involving two CER, CHOL and C24 FFA) to determine how changes in the CER:FFA ratio impact on the structure of the LPP and thereby modify the skins barrier function. More specifically, we seek to determine the relative positions of the ceramide h/c chains and so determine the position and conformation of the CER and FFAs in the model systems used to mimic healthy and diseased SC. The improved understanding so gained of diseased skin will pave the way for improved treatment of these conditions.

Effect of ceramide loss on the lipid ordering within the long periodicity phase of the stratum corneum

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Beamline: D16
Local Contact: Bruno Deme
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Background

The stratum corneum (SC) lipid matrix is critical for the skin's barrier function (1). Composed of primarily ceramides (CERs), cholesterol (CHOL) and free fatty acids (FFAs), these lipids form a long periodicity phase (LPP), which consists of a unique tri-layer unit cell structure, primarily in an orthorhombic phase (2-4).

For the synthesis of these lipids, an enzyme driven pathway is implemented (5). If these enzymes are down- or upregulated as found in inflammatory diseases, the final lipid composition is affected, often altering the barrier function.

Aim of this experiment

In this study, we mimicked the effects of the down regulation of enzymes involved in the synthesis of the sphingosine and measured its effects on the LPP structure. In a simple four lipid LPP model, we substituted CER N-(tetracosanoyl)-sphingosine (CER NS) with FFA C24 and C18 to simulate the loss of the sphingosine headgroup.

These models were then investigated on D16 with complementary studies with FTIR and SAXS to identify if alterations in the LPP had occurred.

Method

The lipid composition used in our model comprised CER EOS, CER NS, CHOL and FFA C24 in a 0.4:0.6:1:1 molar ratio and mimics important aspects of the lipid organization in the SC, including the formation of the LPP and a primarily orthorhombic lateral packing. The CER EOS concentration was increased from native concentrations (around 12%) to 40 mol% of the CER content, to ensure the LPP would form exclusively (6).

Table 1 shows the models measured where the lipid composition was changed by substituting a single CER NS with a FFA C24 and FFA C18 (25% FFA sub). In all models, the carbon chain number and length remained consistent. For the small angle neutron scattering (SANS)

measurements, CER NS-d7, CER NS-d47 and FFA C18-d35 were substituted into the model, replacing their protiated counterparts.

Table 1: LPP models used in this study.

Model	Composition	Ratio
0% Sub	CER EOS:CER NS:CHOL:FFA C24	0.4:0.6:1:1
25% FFA-sub	CER EOS:CER NS:FFA C18:CHOL:FFA C24	0.4:0.45:0.15:1:1.15

Samples were measured at 25°C in the humidity chamber available at D16, with measurement times of 5-6h depending on the signal to noise ratio. Further details on the sample preparation can be found in the published article.

Results

To probe if there was any change in the LPP structure in the 25% FFA-sub models, the lipid arrangement in the unit cell of the LPP was investigated with SANS. The intensity vs q curves for all models demonstrated that the only lamellar structure present was the LPP, with the only additional structure being phase-separated crystalline CHOL. The repeat distances were determined by least square fitting of all peak positions. The mean repeat distance determined for the 25% FFA-sub model was 12.6 ± 0.1 nm.

The location of the deuterated chains was determined by the subtraction of the scattering length density (SLD) profile for the non-deuterated sample from that of the profile for the sample involving deuterated lipid, both hydrated in 8% D_2O solvent. Fig 1 shows the SLD profile for the 25% FFA-sub model. The blue curve shows the location of water at the unit cell boundary at 2.1 nm from the cell centre and at the cell boundaries. The position of the terminal sphingosine chain of CER NS-d7 (green curve) is located 4.2 nm from the centre of the LPP unit cell, while the acyl chain of CER NS-d47 (red curve) is distributed between the central and outer lipid layers of the unit cell. These positions imply that the CER NS in the centre remains in an extended conformation while a proportion of the CER NS is also present in the outer regions. The SLD profile of the free FFA C18 (black curve) shows no regions of higher SLD intensity throughout the unit cell implying the FFA C18 is distributed evenly throughout.

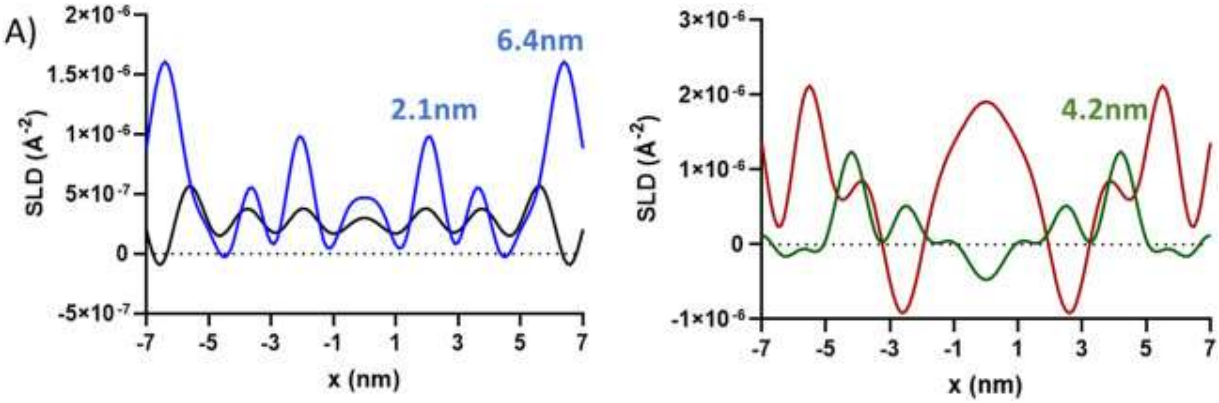

Figure 1: SLD profile of 25% FFA-sub, where 25% of CER NS is substituted with a FFA C24 and FFA C18. A) The water profile (blue) and the position of the entire carbon chain of the FFA C18 (black). B) The terminal position of the CER NS's sphingosine chain (d7, green), and the length of the acyl chain (d47, red).

References

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