Experimental Report

Proposal: **8-02-643** **Council:** 4/2012

Title: Lipid organisation in the stratum corneum

This proposal is continuation of: 8-02-567

Researh Area: Biology

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Samples: ceramides 1 and 2; C16 and C24 fatty acids; cholesterol

Instrument	Req. Days	All. Days	From	To
D16	8	7	26/11/2012	03/12/2012

Abstract:

The lipid matrix in the upper layer of the skin is crucial in establishment and maintenance of its barrier function. These lipid regions comprise cholesterol (CHOL), free fatty acids (FFAs) and ceramides (CERs) forming two crystalline lamellar phases with repeat distances of 5.4 nm and 12.5 nm. In previous studies performed at ILL (8-02-480, 8-02-502 and 8-02-559) we successfully resolved the scattering length density profile of the repeating unit of the 5.4 nm phase, and determined the positions within this repeating unit of the deuterated acyl chain of CER2 and two deuterated moieties of CHOL. We have also determined the scattering length density profile of the repeating unit of the 12.5 nm phase. However, as this phase is very complex, we need more information to construct a sufficiently detailed molecular model. Previously, we performed studies with CER1 having a deuterated linoleic tail. In the scheduled studies in April 2012 we will focus on the position of deuterated CHOL. To obtain a complete picture, the aim of the proposed study is to determine the position of the deuterated chain of CER2 and the deuterated fatty acids in the repeating unit in this phase.

Introduction

The skin is the largest organ of the human body and provides the essential barrier against outer environment. The barrier function of the skin is primarily provided by the lipid matrix of its outermost layer, the stratum corneum (SC)[1-2]. A detailed knowledge of the molecular organisation of the lipids within this matrix is thus central to our understanding not only of the barrier function of healthy skin but also the impaired function in diseased skin and, in consequence, it is also essential to facilitate the rational design and development of transdermal therapeutic agents[1,3].

The SC lipid matrix mainly comprises ceramides (CERs), cholesterol (CHOL) and long chain saturated (C16:0-C24:0) free fatty acids (FFAs). Two crystalline lipid lamellar phases are identified with *d*-spacings of ~6 nm (short periodicity phase, SPP) and ~13.0 nm (long periodicity phase, LPP)[4]. It has been reported that the LPP plays a central role in maintaining the skin barrier[5]. Despite much research over recent years, however, the molecular arrangement of the SC lipids within the repeating unit of the lamellar phases is still unknown. In our own work in this area, we have found that the determination of electron density profiles from X-ray data has proved particularly problematic (because of the lack of any significant swelling on hydration of the lamellae)[6,7]. In the studies conducted here, our aim was to employ the technique of neutron diffraction in conjunction with H/D contrast variation (afforded through the use of selectively deuterated lipids) to furnish the neutron scattering length density profile of the lipid lamellae, and allow the key lipid components to be located within the SC matrix.

From our previous experiment, we were able to determine the molecular position of CHOL and FFA C24 in the SPP system[8]. Therefore in this experimental session, we focussed on the LPP system. Our system comprised of six CERs (including long chain CERs also known as acyl CERs)/CHOL/FFA7 and formed only the LPP (with 13 nm d-spacing). Our aim here was to begin our studies using this simple model system, which is nevertheless very good models for native SC, in order to facilitate our interpretation of the neutron diffraction data.

Sample preparation

All the samples were prepared by dissolving the lipid mixtures in chloroform/methanol (2:1, v/v) and then spraying under a continuous nitrogen stream over an area of 4.8 cm^2 on silicon substrates. Samples were then equilibrated for 10 minutes at a temperature of 70°C, and subsequently cooled down to room temperature and hydrated for 12 hours at 37°C.

Data collection & analysis

Data were collected with an omega scan of 0 to 13 degree to cover the first nine diffraction orders, with samples of varying contrast, hydrated at 3 different H_2O/D_2O atmospheres. In order to determine the localization of the deuterated materials, we prepared 4 different set of samples: CHOL tail deuterated, CHOL head deuterated, CER NS deuterated and free fatty acid C24 deuterated.

Results & Conclusions

We are still analysing the data from our LPP measurements. The data looks promising so far. An example of the samples without any deuteration is shown below-

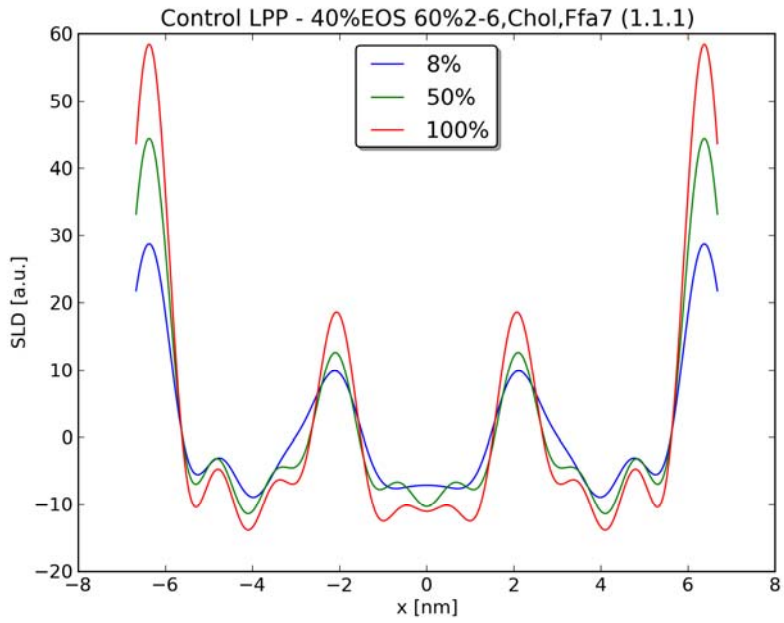

Figure: Neutron scattering length density (SLD) profiles of model lipid membranes in the direction normal to the unit cell surface. Above figure displays LPP system without any deuteration and acts as control. The contrast difference at different hydration buffer indicates the sample reproducibility and instrument functionality.

Currently, we are able to determine the intensities, structure factors of the LPP system containing deuterated CER NS and FFA C24. We are now working on determining the water phase sign in the LPP system. One promising phase sign so far is -+-+-+-+- for the first 9 diffraction orders and the profile generated with this phase sign was shown above. We have some preliminary information about the CHOL positioning in the LPP system as from the experiment and using the above phase sign. The experimental results indicate that CHOL molecule is located with their head group near the inner high density part of the lipid head group and the tail approaching to the outer border of the unit cell.

References

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