Experimental report

06/06/2017

Proposal: 8-02-788 **Council:** 10/2016

Title: Lamellar neutron diffraction studies of a simplified (4-component) model stratum corneal system

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Ceramide-1:Ceramide-2:Cholesterol:C24:0 fatty acid
Ceramide-1:Ceramide-3:Cholesterol:C24:0 fatty acid

Instrument	Requested days	Allocated days	From	To
D16	7	6	24/02/2017	02/03/2017

Abstract:

The lipid matrix in the upper layer of the skin is crucial to the skin’s barrier function. This matrix contains cholesterol (CHOL), free fatty acids (FFAs) and ceramides (CERs), arranged as lamellar phases with repeat distances of ~5.4 nm (SPP) and ~12.5 nm (LPP). In previous studies performed at ILL, we successfully resolved the scattering length density profiles of the SPP and LPP, and subsequently determined the positions within the LPP phase of the FFA, CER2 acyl chain, CHOL, and CER 1 linoleate. The LPP studied previously was prepared from a mixture of six CERs, CHOL, and FFA, and was found to have a complex structure involving three lipid layers, with water at the boundaries of the unit cell and in the interior. In recent X-ray diffraction studies, we have now established that the LPP can be prepared using only 4 components, with CER1 in combination either with CER2 or CER3. However, the repeat distance varies between 12.4 and 13.6 nm, and so it is uncertain whether the CERs and CHOL are arranged as found within the 8-component, three-layer LPP. In this proposal we will focus on the simplified 4-component mixture and determine the positions of the CER2, CER3, and CER1.

Investigation of the Behavior of a Simple Stratum Corneum Lipid Mimicking Model, and the Position of Each Component

Proposal: 8-02-788
Beamline: D16
Local contact: Bruno Demé
Experiment date: 24th -1st March 2017

Background

The skin's barrier function is provided by the stratum corneum (SC), which consists of the upper 20 µm layer of the skin. The structure of the SC is often compared to a brick wall – with dead cells serving as the bricks and the extracellular lipids acting as the mortar.
The SC lipid matrix is mainly composed of cholesterol (CHOL), free fatty acids (FFAs), and ceramides (CERs). These lipids can form two lamellar phases with periodicities of 5.4 nm (the short periodicity phase, SPP) and 12.5 nm (the long periodicity phase, LPP)[1]. Previous studies have revealed that it is crucial for several different lipid classes to be present such as CER EOS and CHOL in order to form the LPP[2-4].
Previous neutron diffraction studies performed at D16, have shown sufficient H_2O/D_2O contrast variation to determine the scattering length profile of the SPP and the LPP. In addition, the localization of the deuterated moieties in the repeating unit of the lamellar phases have been determined [5-9].
Recently the LPP was formed consisting of only two CER groups (CER EOS and CER NS) that were mixed in equal ratios with CHOL and FFA5 (palmitic acid (C16), steric acid (C18), arachidic acid (C20) , behenic acid (C22), lignoceric acid (C24) in molar ratios of 1.8:4:7.6:47.8:38.8). A previous X-ray experiment had shown the repeat distance and the intensity distribution of the diffraction peaks where similar to that of the previous model. However, it is not clear whether the arrangement and localization of the CER, FFA and CHOL would match the more complex system consisting of many different subclasses of CER and FFA.

Aim of this Experiment

During this experiment we aimed to investigate if the simpler two CER SC lipid model was arranged similarly to the more complex models that have been previously investigated. We aimed to determine the phase behavior of lipids at skin relevant temperatures (32 °C), as well as deuterating each component of the system individually in order to identify their position within the unit lattice.

Method

Five samples were measured during this experiment, the compositions are shown below. Components in bold were deuterated:

- CER EOS/CER NS:CHOL:FFA5
- CER EOS/**CER NS:**CHOL:FFA5

- CER EOS/CER NS:**CHOL d6 (head)**:FFA5
- CER EOS/CER NS:**CHOLd7 (tail)**:FFA5
- CER EOS/CER NS:CHOL:**FFA5**

Samples were prepared in an 2:1 chloroform:methanol solution and sprayed on silica wafers. Once sprayed the lipids were mixed by 2 equilibration cycles that consisted of slowly heating the sample until fully melted, holding for 10 minutes at temperature, and then slowly cooling back to room temperature. The samples were stored under argon until the start of the experiment. Before measuring each sample was hydrated for at least 11 hours at 35 °C, to ensure that the sample was fully hydrated.
The samples were measured at D16 using the humidity chambers. The chambers were heated to 32 °C at a relative humidity 100%. Three different solvent contrasts were measured (8, 50, 100%) in order to fit the structure factor of the samples and identify the water distribution.

Results

During this experiment, we did not observe the higher order Bragg peaks in any of the samples. In the case of the fully protonated system shown in Figure 1, the measurements at the respective 2theata (Ω) ranges showed no Bragg peaks beyond the cholesterol peak at Ω = 7.7°. After selecting the relevant scans to increase the resolution of each of the Bragg peaks only the first 3 peaks (and CHOL) could be identified in all of the samples. Due to lack of higher order peaks, we were unable to identify the phase and the water profile of the samples, as well as the position of the components in the deuterated samples.

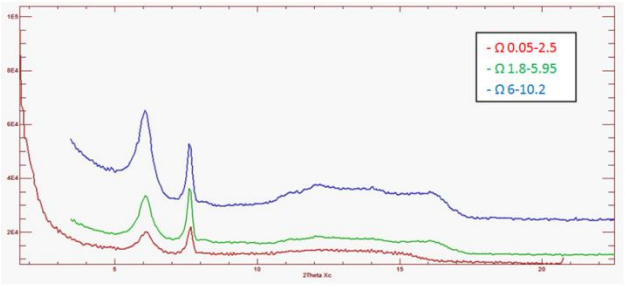

Figure 1 Spectra for the fully protonated (CER EOS/ CER NS (60/40)):CHOL:FFA5 1:1:1 hydrated in 50:50 H2O:D2O at 100% hydration. For highest resolution, measurements between Ω 0.05-2.5 were measured at gamma (γ) of 11.2 while 1.8-5.95 was measured at γ 13. Ω 6-10 was also measured at γ 13 but the measuring time was increased from 90s to 120s to accommodate the weaker scattering at the higher orders.

Discussion

During previous experiments, our group has been successful at collecting spectra with a large number of Bragg peaks at the D16 station. Figure 2 is a scan obtained from a similar lipid sample measured during a previous experiment in 2011 at D16. The Bragg peaks in Figure 2 are visible up to Ω ~20°, while in the case of this experiment, on average, Bragg peaks did not exceed the third order (Ω ~6-7°, Figure 1). Note the peak between 7 and 8° in Figure 1 & 2 is the CHOL peak, and is not part of the lamellar structure.

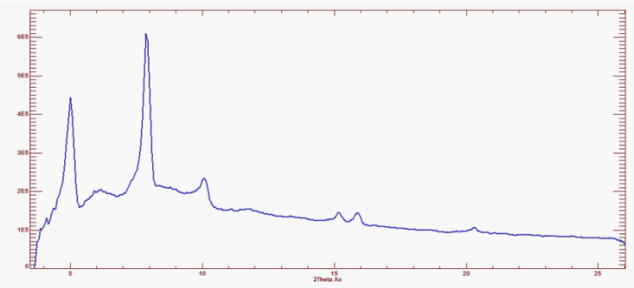

Figure 2 1D scan of CER 2-6:CHOL D7:FFA7 1:1:1 sample measured at D16 in July 2011. 7 peaks (including the cholesterol peak) can be observed between Ω of 5 - 20°.

Complementary small and wide angle X-ray diffraction (SAXD and WAXD) measurements were performed after transferring the same measured samples to mica sheets and measured on the BM26 beamline at the ESRF (May 2017). Figure 3 shows the x-ray scan of the same sample measured in Figure 1. During this measurement, 6 Bragg orders (solid blue arrows) were observed.

These results implies that the experiential measurements at D16 may not have been optimal. Discussion with the beam line scientist on how to improve our sample measurements for the future are in progress.

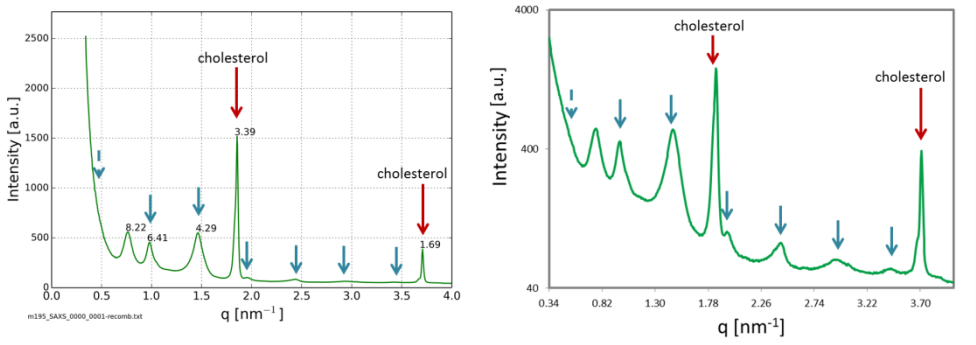

Figure 3 1D scans of recent SAXD measurements at the BM26 beam at the ESRF with intensity both linear (left) and logged (right). Two reflections for the cholesterol was observed (red arrows) as well as six reflections (blue solid arrows) missing the first bragg peak (blue dotted arrow).

References

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