Experimental report

01/03/2019

Proposal: 8-02-806 **Council:** 4/2017

Title: Neutron diffraction studies of stratum corneal lipid lamellae models:determination of ceramide locationand conformation

Research area: Biology

This proposal is a new proposal

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Samples: ceramide-1/ceramide-2/cholesterol/C24 fatty acid
ceramide-1/d7-ceramide-2/cholesterol/C24 fatty acid
ceramide-1/d47-ceramide-2/cholesterol/C24 fatty acid
ceramide-1/d7-ceramide-2/ceramide-3/ceramide-4/ceramide-5/ceramide-6/cholesterol/C24 fatty acid
ceramide-1/ceramide-2/ceramide-3/ceramide-4/ceramide-5/ceramide-6/cholesterol/C24 fatty acid
ceramide-1/d47-ceramide-2/ceramide-3/ceramide-4/ceramide-5/ceramide-6/cholesterol/C24 fatty acid

Instrument	Requested days	Allocated days	From	To
D16	6	6	03/04/2018	09/04/2018

Abstract:

The lipid matrix of the skin contains cholesterol (CHOL), free fatty acids (FFAs) and ceramides (CERs), arranged as lamellar phases with d-spacings of ~5.4 nm (SPP) and ~12.5 nm (LPP). In previous studies at ILL, we successfully resolved the scattering length density profiles of the SPP and LPP, and determined the positions within the LPP of the FFAs, CER-2 acyl chain, CHOL, and CER-1 linoleate. The LPP system studied previously involved an 8-component mixture, and was found to have a complex structure involving three lipid layers, with water at the boundaries and interior of the unit cell. We have now established through X-ray studies that the LPP can be prepared using just CER-1, CER-2, CHOL, and FFAs. However, the repeat distance in this system is not the same as in the 8-component LPP system, and so it is not known if these two systems are structurally equivalent. Moreover, we still do not know whether the CER-2 adopts a hairpin or fully extended conformation. Here, we aim to use CER-2 with a d7-sphingosine tail, to allow unambiguous determination of CER-2 conformation, and also to determine if the arrangement of CER-2 differs between the complex and simple LPP model systems.

Neutron diffraction studies of stratum corneal lipid lamellae models: determination of CER NS location and conformation

Proposal: 8-02-806
Beamline: D16
Local contact: Bruno Demé
Experiment date: 2nd -9th April 2018

Background

The skin lipid matrix is the structure used for materials to translocate through the skin barrier, and thus of great interest for drug deliver and skin diseases. The lipid matrix mainly contains three types of lipids in an equimolar ratio, namely: cholesterol (CHOL), free fatty acids (FFA) and ceramides (CERs). These lipids arrange to form 2 distinct lamellar structures, referred to as the short and long periodicity phases (SPP & LPP). In the LPP, the lipids are primarily arranged in a tri-layered lamellar structure with a repeat distance of 13nm, this structure is unique to the SC layer and is assumed to play an important role in the barrier function.
To identify the behavior of the lipid matrix several models have been developed, which range from the highly complex, containing many different lipid subclasses, to the very simple, which contain fewer than 10 lipid subclasses. The advantages of these simplified models enables studies into the interactions between lipid subclasses [1] as well as predictive models for permeability [2]. These simple models can also accommodate deuterated lipids, thus enabling in-depth SANS studies. In contrast, the more complex models mimic the lipid composition found in native skin to a greater extent. This is important to determine that these different LPP models exhibit similar lipid arrangements. Previous neutron studies have identified the position of specific lipid subclasses in simple models of the LPP phase, however the conformation of the lipid subclasses remains understudied.

Aim of this Experiment

During this experiment we aimed to investigate the location and the conformation of the crucial CER non-hydroxy sphingosine (NS) lipid, within the LPP structure. This involved determining the location of each of the CER chains within the LPP's trilayer structure.

Method

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

- CER EOS/CER NS:CHOL:FFA(5 chain lengths)
- CER EOS/**CER NS-d7:**CHOL:FFA(5 chain lengths)
- CER EOS/**CER NS-d47:**CHOL:FFA(5 chain lengths)
- CER MIX/CER NS:CHOL:FFA(7 chain lengths)
- CER MIX/**CER NS-d7**:CHOL: FFA(7 chain lengths)

- CER MIX/**CER NS-d47**:CHOL: FFA(7 chain lengths)

Samples were prepared in an 2:1 chloroform:methanol solution and sprayed on silicon wafers. Once sprayed the lipids were mixed by 2 equilibration cycles that consisted of slowly heating the sample until fully melted, holding for a combined total of 30 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 15 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

The contrast variation using deuterated lipids revealed that the LPP consist of trilayered structure with headgroup regions in between: one central layer and two boundary layers (Figure 1, blue curve). Subsequently, the number, and signal/noise ratio of the Bragg peaks were sufficient enough for determine the scattering length densities (SLD) for all of the samples mentioned above. The location of each of the deuterated sections of the CER NS lipids were identified, with the acyl chain concentrated in the central region and the sphingosine chain concentrated in the other layer of the LPP. Implying the molecule was in a linear conformation (Figure 1).
In addition, we found the repeat distance of the LPP was slightly shorter than the typical 13 nm found in native skin (~12nm), while modeling the arrangement that CER chain that appeared to have adopted to tilt in order to accommodate this arrangement. Full calculation of the SLD profiles and detailed analysis are in progress.

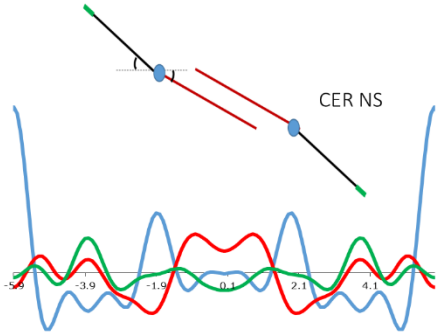

Figure 1 Preliminary SLD profile for CER NS's acyl chain (red) and sphingosine chain (green). The acyl chain is concentrated in the center of the LPP structure, while the sphingosine chain is concentrated in the middle of the outer leaflets. LLP unit cell length was 12.1 nm, the location of water molecules are shown.

Conclusions

The results from this experiment demonstrate that CER NS adopts an extended conformation. This observation was see in both the simplistic and the more realistic model, demonstrating the repeatability of these models. Once the data analysis is completed , the results combined with an experiment performed at ISIS neutron source (UK) will be sufficient to construct a publishable manuscript.

References

1. Mojumdar, Enamul H., et al., *Skin Lipids: Localization of Ceramide and Fatty Acid in the Unit Cell of the Long Periodicity Phase.* Biophysical Journal, 2015. **108**(11): p. 2670-2679.

2. de Jager, M., et al., *A Novel in Vitro Percutaneous Penetration Model: Evaluation of Barrier Properties with P-Aminobenzoic Acid and Two of its Derivatives.* Pharmaceutical Research, 2006. **23**(5): p. 951-960.