Experimental report

18/02/2022

Proposal: 9-13-902 **Council:** 10/2019

Title: A complex model of skin lipid barrier from isolated human stratum corneum lipids

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Chol/HumanSkinFFA/HumanSkinCer
d4-Chol/HumanSkinFFA/HumanSkinCer
HumanSkinChol/FFA(16-24)/HumanSkinCer
HumanSkinChol/d47-Tetracosanoic acid/FFA(16-22)/HumanSkinCer
HumanSkinChol/HumanSkinFFA/CerEOS/CerNS/HumanSkinCer(except CerNS and CerEOS)
HumanSkinChol/HumanSkinFFA/CerEOS/d47-CerNS/HumanSkinCer(except CerNS and CerEOS)

Instrument	Requested days	Allocated days	From	To
D16	5	5	18/08/2020	21/08/2020
			22/03/2021	24/03/2021

Abstract:

The stratum corneum (SC) prevents the dehydration of terrestrial mammals and hampers the entry of possibly harmful substances through the skin. The SC barrier lipids are cholesterol (Chol), free fatty acids (FFA), and ceramides (Cer). They are organized in a repeating sequence of broad/narrow/broad electron-lucent bands bordered by electron-dense segments with a repeat distance d = 13 nm. Skin diseases are linked with the permeability barrier dysfunction and an aberrant lipid organization. The aim of the proposal is to reconstruct the extracellular domains of SC using isolated purified human skin lipids and to determine its molecular arrangement. The individual lipid classes (or groups of the classes) will be separated by column chromatography and selectively replaced by protonated or deuterated synthetic lipids. The molecular organization of the complex model will be compared with the previously measured simple SC model based on 2 synthetic Cer. The characterized complex model can serve for further studies of the interaction of pathophysiological or therapeutic molecules with SC lipid matrix.

A complex model of skin lipid barrier from isolated human stratum corneum lipids

Introduction

The stratum corneum (SC) barrier is an evolutionary adaptation of mammals, including humans, to terrestrial life. SC prevents the desiccation of organisms because it limits the loss of water in the environment. SC also hampers the entry of potentially harmful substances into the organism. The barrier function of SC is maintained by extracellular lipids forming a repeating sequence of broad/narrow/broad electron-lucent bands bordered by electron-dense segments[1]. The main SC lipids are ceramides (Cer), free fatty acids (FFA) and cholesterol (Chol)[2]. Ultra-long ω-*O*-acylCer (EO-Cer), which contain 30-34C acyls with linoleic acid ester-linked to ω-hydroxyl, play a crucial role in the homeostasis of the skin lipid barrier[3]. The molecular organization of extracellular SC lamellae remains under discussion[1, 4-6].

The aims of the experiment were:
1. to develop an SC model with a high degree of complexity similar to the native SC, i.e., the complex SC model.
2. to explore the molecular arrangement of the complex SC model, particularly of the long lamellar phase providing the repeat distance (*d*) of ~ 13 nm and to compare it with the simple SC model composed of synthesized lipids.

Experiment

The complex model was developed from the isolated human skin barrier lipids. The abdominal or breast skin was obtained from the Caucasian female patients, who had undergone plastic surgery and had given their written informed consent. The procedure was approved by the Ethics Committee of Sanus First Private Surgical Centre, Czech Republic, and was conducted according to the principles of the Declaration of Helsinki. Free SC lipids were extracted with a sequence of chloroform/methanol solvents. The extracted lipids were purified and separated to several fractions by column chromatography and their composition was verified using high-performance thin-layer chromatography.

We proposed to prepare the complex SC models containing i) isolated purified human skin lipids (HSL), ii) HSL mixed with synthesized protonated lipids, and iii) HSL mixed with perdeuterated synthesized lipids (i.e. parallel samples with protonated and perdeuterated lipid species). All the models were prepared, but those, in which a specific lipid fraction was replaced by synthesized lipids, did not form a regular structure of sufficient quality to measure neutron diffraction (ND). Advanced research would be needed to reveal the phase behavior of the mixed HSL/synthesized lipids complex SC models.

For ND, six individual samples of the complex SC lipid model were prepared from different batches of HSL. Eight samples composed of synthesized lipids were prepared as backup samples. Five samples were measured under constant relative humidity (RH) conditions or under gradually increasing RH. We gratefully announce the help of Dr. Demé with the measurement because the experiments were performed without the presence of users in 2020 and 2021. The ND patterns of the complex SC lipid model (panel A) and the simple synthesized lipid model (panel B) are shown in Figure 1.

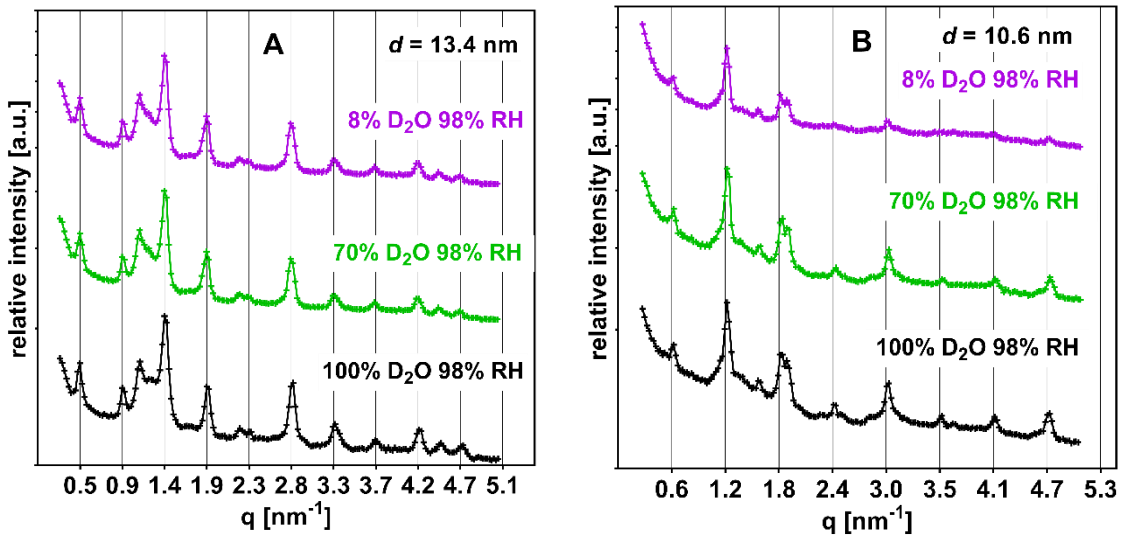

Figure 1: The ND patterns of the complex SC lipid model (panel A) and synthesized lipid model (panel B) at indicated relative humidity (RH) and contrast (%D_2O) conditions. Grid lines predict the position of diffraction orders at the given repeat distance (RD).

We have determined the repeat distance of the lamellar stacking of 13.4 nm and 10.6 nm in the complex SC lipid model and the simple synthesized lipid model, respectively. The relative neutron scattering length density (NSLD) profiles were reconstructed from 10 diffraction orders for the complex SC lipid model and 8 diffraction orders for the simple synthesized lipid model at different contrast conditions (D_2O/H_2O ratio). Furthermore, the water distribution and Patterson functions were calculated for both models. The relative NSLD of samples measured at gradually increasing RH will be reconstructed by using the phase angles determined in the contrast variation experiment.

Conclusions

The ND results obtained in the experiment enable the comparison of the lamellar arrangement formed by the isolated purified human skin lipids with d = 13.4 nm and the simple model based on synthesized lipids with d = 10.6 nm. The ND results are corroborated with small-angle X-ray scattering data of the complex SC model, which are suitable to reconstruct the relative electron density profiles of the models. Furthermore, we aim to obtain the density profiles across human SC specimens from electron microscopy to support our analyses of diffractometric data.

References

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