

Experimental report

18/08/2023

Proposal: 9-10-1739

Council: 10/2022

Title: Introducing a new ceramide subspecies to ternary Stratum corneum lipid model membranes: CER[NH]

Research area: Biology

This proposal is a new proposal

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Samples: CER[NP]-C24/CHOL/LA
D-CER[AP]-C24/CER[NP]-C24/CER[NH]-C24/CHOL/LA
D-CER[AP]-C24/CHOL/LA
D-CER[AP]-C24/CER[NP]-C24/CHOL/LA
CER[NH]-C24/CHOL/LA
CER[NH]-C24/D-CER[AP]-C24/CHOL/LA
CER[NH]-C24/D-CER[AP]-d3/CHOL/LA

Instrument	Requested days	Allocated days	From	To
D16	5	5	15/06/2023	20/06/2023

Abstract:

All Substances, which want to overcome the skin with the aim to leave or to penetrate into the human body, have to pass the skin's main barrier, the stratum corneum (SC). Thereby the structural organization of the SC lipids and especially their function in the lipid lamellae play a key role. The heterogeneous group of ceramides (CER), as the main component of the lipid matrix, offers a wide range of influence possibilities. Especially a ternary CER mixture and its impact to the lamellae forming process and to the barrier function is still an immense point of interest.

With the description of a new short-chain CER, a substantial interest in its arrangement within the lipid multilayer system on a molecular level and additionally its influence to the skin's retarding effect was born. As a part of my habilitation treatise this experiment is very important for the basically understanding of SC lipid arrangements on molecular level.

"Introducing a new ceramide

subspecies to ternary Stratum corneum lipid model membranes: CER[NH]".

Introduction

As the barrier properties of the human skin are mostly relating to the arrangement of the lamellar lipids in the outermost layer, the so-called Stratum corneum (SC) [1], it became the focus of interest in the last decades. However, the lipid lamellae is not only connected to the barrier properties but is the main penetration route for penetrating substances as well [2]. So the correlation between the lipid arrangement and the barrier properties is an immense point of interest. Here, model membranes can be investigated to avoid the complexity of native lipids. Based on native lipid ratios, realistic models with the SC lipid classes of ceramides (CER), cholesterol (CHOL) its sulfate salt (ChS) and free fatty acids (FFA) can be used. The group of CERs is very heterogeneous, as overall to this date, 20 subclasses are described [3-6]. For more detailed insights into the structure-function relationship of the lipids along each other, a new CER subclass together with CHOL and lignoceric (LA) was introduced for the first time in a neutron diffraction experiment. A deuteration of the amid-bound C24-fatty acid of the CER was a gainful feature in this neutron diffraction experiment for the localization of the chain within the membranes.

The experiment

The samples

The following samples were measured with a mass ratio of 55/25/15/5 to compare it to prior experiments implying this system [7, 8]:

CER[NH]-C24_CHOL_LA_ChS

CER[NH]-C24-d₄₇_CHOL_LA_ChS

The structures of the appropriated lipids are presented in Fig.1:

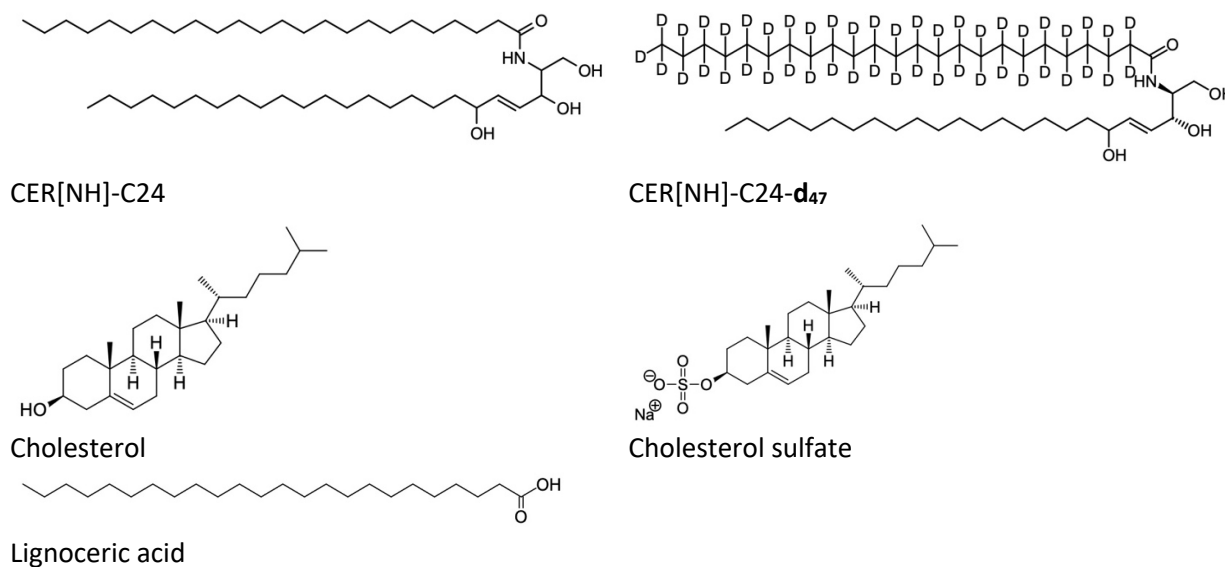


Figure 1: Scheme of the applied lipids. CER[NH] was applied as protonated (t18:1/24:0) and perdeuterated variants next to cholesterol (CHOL), the C24 FFA lignoceric acid (LA) and cholesterol sulfate (ChS).

Membrane structure investigations

All measurements were performed for better internal comparableness with the same omega range from 0-20° relating to the Bragg peaks positions of the samples. Constant temperature and relative humidities (RH) were realized by three cans whereby an online temperature regulation of the sample was possible. Water baths were used for the temperature regulation, put into effect of the skin temperature of 32 °C. The samples were measured at 98% RH and for a contrast variation at 8, 50 and 100 % D₂O. Due to a limited signal-to-noise ratio of the samples, each measurement was performed for about 7 h for a better statistic of the received data.

First results

Due to the new data treatment software MANTID, we have to create new devices and tools in our data analyse software, which takes more time than expected. However, a few raw data could be extracted already and been presented here. It was possible to obtain well-oriented Bragg peaks up the 7th order. The received scattering patterns of V11 and V12 (100 % D₂O, 98 % RH, 32 °C) are presented in Fig. 2. It has to be noticed, that no background subtraction was done jet.

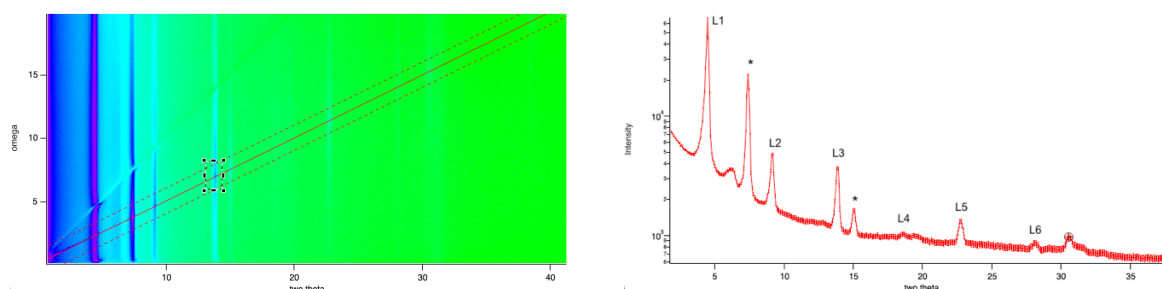


Figure 2: Neutron scattering pattern of CER[NH]-d47_CHOL_LA_Chs (left) and its plotted peak (right). At a detector position of 43.5°, up to 6 orders were visible (L1-L6). Furthermore crystalline CHOL peaks were detected (asterisk mark).

References

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