Experimental report

17/06/2020

Proposal: 9-13-825 **Council:** 10/2018

Title: The molecular organization of long-periodicity phases in the skin lipid barrier models

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Petra PULLMANNOVA

Experimental team: Elena ERMAKOVA
Norbert KUCERKA
Petra PULLMANNOVA
Veronika SOMMEROVA

Local contacts: Bruno DEME

Samples: CerNS24/FFA(16-24)/Chol/CholS
d47-CerNS24/FFA(16-24)/Chol/CholS
CerEOS/CerNS24/FFA(16-24)/Chol
CerEOS/d47-CerNS24/FFA(16-24)/Chol

Instrument	Requested days	Allocated days	From	To
D16	4	4	20/06/2019	24/06/2019

Abstract:

The extracellular lipids of stratum corneum (SC), which are organized in a lamellar pattern, prevent the dehydration of terrestrial mammals and hamper the penetration of exogenous substances through the skin. These lipids contain mainly ceramides, free fatty acids and cholesterol (Chol) mixed in an equimolar ratio. The repeating unit with the d = 13 nm is a physiologically important feature of the skin barrier, but its molecular arrangement is still under debate. An impaired skin barrier function and aberrant skin lipid organization occur in skin diseases, e.g. in recessive X-linked ichthyosis (RXLI). We prepared the simplified lipid model of RXLI with the increased fraction of skin Chol precursor - cholesteryl sulfate (CholS) at the expense of Chol. This model showed unusual polymorphic behavior modulated by the CholS content and sample annealing method. The sample with high CholS content can form a lamellar phase with the d = 10.6 nm similar to the d found in SC. The aim of the proposed study is to investigate the molecular arrangement of the lamellar phase modulated by CholS by neutron diffraction. This phase will be compared with the model of long repeat unit found in the skin.

The molecular organization of long-periodicity phases in the skin lipid barrier models

Introduction

The aim of the experiment was to reveal the molecular organization of the models of stratum corneum (SC) lipid matrix. The main SC extracellular lipids are ceramides (Cer), free fatty acids and Chol in an approximately equimolar ratio[1]. One of the minor components of the SC lipid matrix is cholesteryl sulfate (CholS). Its normal SC level is up to 5 wt.% of SC lipids, but it is pathophysiologically increased in the recessive X-linked ichthyosis (RXLI)[2]. The SC lipid matrix is ordered in a regular pattern of broad/narrow/broad electron-lucent bands bordered by electron-dense segments[3]. The SC models based on isolated human skin lipids or synthetic lipids attain the regular arrangement providing the long repeat distance of ~ 13 nm, but also the short repeat distance of ~ 5 – 6 nm and crystalline Chol detectable by X-ray diffraction (XRD)[4]. The presence of ultralong ω-*O*-acylceramides (EO-class Cer) is essential for the formation of the SC lamellar structure[5].

The increased level of CholS in the RXLI model based on CerNS (without EO-class Cer) leads not only to its partial phase separation but also facilitates the formation of another phase – medium lamellar phase (MLP) with the repeat distance of 10.6 nm[6]. Previous experiments showed that MLP can be formed also by the CerNH24 based model membranes[7]. We aimed to compare the molecular organization of MLP with the molecular organization of the long lamellar phase (d = 12.2 nm) formed due to the EO-class Cer in a model of the healthy SC. The structure of similar SC models was previously studied by neutron diffraction experiments[8-13] and the data were evaluated as a centrosymmetric membrane with water penetrated the hydrophilic region of the bilayer.

Experiment

For the neutron diffraction experiment, the proposed samples were prepared along with the additional back-up samples. Ultra-long ceramide CerEOS (EO-class Cer), very long-chain CerNH24 and partially deuterated d_{47}-CerNS24 and d_{47}-CerNH24 were synthesized at the Faculty of Pharmacy in Hradec Králové, Charles University, Prague. d_4-Chol was obtained from C/D/N Isotopes Inc., Quebec, Canada. Other components of the SC model membranes were purchased from Avanti Polar Lipids (Alabaster, USA) and Sigma-Aldrich Chemie, Gmbh (Schnelldorf, Germany).

The lipids were mixed with a respect to the native ratios of human skin lipids, but with decreased Chol level to diminish its phase-separation. To mimic the pathophysiology of RXLI, we used sphingosine-based CerNS24-subclass mixed with FFA (16:0-24:0), Chol and CholS at a 1:1:0.5:0.5 molar ratio. The analogous samples contained either deuterated d-$_{47}$CerNS24 or deuterated d_4-Chol instead of protonated molecules. The long lamellar phase as a model of the healthy SC lipid barrier was created from CerEOS, CerNS24, FFA (16:0-24:0) and Chol at the molar ratio 0.3:0.7:1:0.45. The analogous sample contained deuterated d-$_{47}$CerNS24 instead of protonated CerNS24. The dissolved lipid mixtures were dropped on the wafers and annealed at 70 °C at the presence of H_2O. CerNH24 or d_{47}-CerNH24 mixed with CerNS24, LIG, Chol and CholS served as a back-up sample that formed a similar MLP structure. The periodical structure of the samples was confirmed by XRD.

Finally, 6 samples were successfully measured at 4 different contrast conditions (100%, 70%, 40%, and 8% D_2O) at 100% relative humidity and T=32 °C to determine the scattering phases. The sample with CerEOS/CerNS24 was not measured because of its high

mosaicity. Generally, the SC lipid model is more rigid and much less hydrated than phospholipid bilayer in line with its ability to limit the water loss of the terrestrial mammals. A limited amount of D_2O/H_2O penetrates the hydrophilic regions of the structure. Therefore, we observed more reflection orders than it is usual for phospholipid bilayers but with lower intensity. The low sample mosaicity and good orientation are crucial for successful measurement. Some neutron diffraction spectra were complicated by the peak convolution. An additional phase with the repeat distance ~ 5 nm occurred in neutron diffraction (ND) pattern, although XRD detected it neither before nor after the experiment at ILL. Therefore, the deconvolution was needed for 2nd order reflection of the MLP phase.

The obtained repeat distances in 100% D_2O were:
CerEOS/d_{47}-CerNS24/FFA(16:0-24:0)/Chol: 12.38 nm (an example of the pattern is in Figure 1)
CerNS24/FFA(16:0-24:0)/Chol/CholS: 10.66 nm
d-$_{47}$CerNS24/FFA(16:0-24:0)/Chol/CholS: 10.69 nm
CerNS24/FFA(16:0-24:0)/d_4-Chol/CholS: 10.66 nm
CerNH24/CerNS24/LIG/Chol/CholS:10.84 nm
d_{47}-CerNH24/CerNS24/LIG/Chol/CholS:10.88 nm

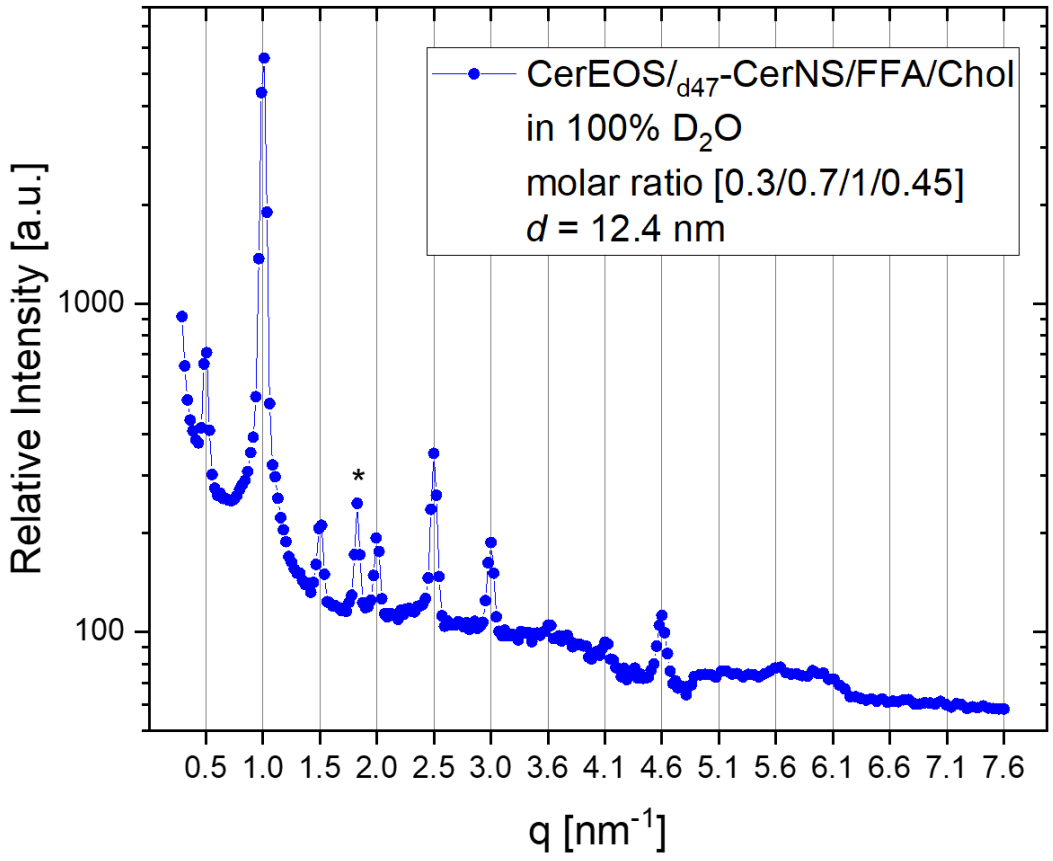

Figure 1: ND pattern measured at 100% D_2O and 100 % relative humidity (RH). The grid lines predict the position of regularly spaced reflections (d = 12.38 nm), asterisk indicates the peak of separated Chol.

Conclusion

The results are in a good agreement with the repeat distances we determined previously using the X-ray diffraction measurements[6]. Such information however reveals the inter-lamellar distances only. The neutron diffraction experiments could assist us in reconstructing the scattering profiles of our SC models. Nonetheless, the non-linear behavior of scattering form factors obtained at various contrast conditions complicates the profile reconstruction. It is intriguing to note here, that the observed non-linearity may point to the asymmetrically organized structures. Recently, solid-state ^{2}H NMR results corroborated by Fourier transformed infrared spectroscopy and X-ray diffraction confirmed the existence of asymmetric molecular assemblies, which may organize in a more complicated manner resulting in periodically arranged asymmetric structures[14].

References

1. P. W. Wertz, *Exogenous Dermatology*, 2004, **3**, 53-56.
2. M. L. Williams and P. M. Elias, *J Clin Invest*, 1981, **68**, 1404-1410.
3. D. C. Swartzendruber, P. W. Wertz, D. J. Kitko, K. C. Madison and D. T. Downing, *Journal of Investigative Dermatology*, 1989, **92**, 251-257.
4. J. A. Bouwstra, G. S. Gooris, M. A. S.-d. Vries, J. A. van der Spek and W. Bras, *International Journal of Pharmaceutics*, 1992, **84**, 205-216.
5. J. A. Bouwstra, G. S. Gooris, F. E. R. Dubbelaar, A. M. Weerheim, A. P. Ijzerman and M. Ponec, *Journal of Lipid Research*, 1998, **39**, 186-196.
6. P. Pullmannová, E. Ermakova, A. Kováčik, L. Opálka, J. Maixner, J. Zbytovská, N. Kučerka and K. Vávrová, *Journal of Lipid Research*, 2019, **60**, 963-971.
7. A. Kováčik, M. Šilarová, P. Pullmannová, J. Maixner and K. Vávrová, *Langmuir*, 2017, **33**, 2890-2899.
8. D. Groen, G. S. Gooris, D. J. Barlow, M. J. Lawrence, J. B. van Mechelen, B. Demé and J. A. Bouwstra, *Biophysical Journal*, 2011, **100**, 1481-1489.
9. E. H. Mojumdar, D. Groen, G. S. Gooris, D. J. Barlow, M. J. Lawrence, B. Deme and J. A. Bouwstra, *Biophysical Journal*, 2013, **105**, 911-918.
10. E. H. Mojumdar, G. S. Gooris, D. Groen, D. J. Barlow, M. J. Lawrence, B. Demé and J. A. Bouwstra, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 2016, **1858**, 1926-1934.
11. M. A. Kiselev, E. V. Zemlyanaya, N. Y. Ryabova, T. Hauss, L. Almasy, S. S. Funari, J. Zbytovska and D. Lombardo, *Appl. Phys. A*, 2013, **116**, 319-325.
12. A. Schroter, D. Kessner, M. A. Kiselev, T. Hauss, S. Dante and R. H. H. Neubert, *Biophysical Journal*, 2009, **97**, 1104-1114.
13. A. Ruettinger, M. A. Kiselev, T. Hauss, S. Dante, A. M. Balagurov and R. H. H. Neubert, *Eur Biophys J*, 2008, **37**, 759-771.
14. O. Engberg, A. Kováčik, P. Pullmannová, M. Juhaščik, L. Opálka, D. Huster and K. Vávrová, *Angewandte Chemie International Edition*, 2020, doi.org/10.1002/anie.202003375.