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E x p e r i m e n t a l R e p o r t

The role of EO-class ceramide in the arrangement of the stratum corneum model membranes

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Introduction

The performed experiment dealt with the model of stratum corneum (SC) extracellular lipid matrix, which comprises ceramides (Cer), free fatty acids (FFA) and cholesterol (Chol) – in approximately equimolar fractions, with a minor amount of cholesteryl sulfate (CholS)[1]. These skin lipids create a highly organized lamellar structure with a repeat distance ~ 13 nm (so-called long periodicity phase)[2] and provide a barrier against water loss and the entering of exogenous substances through the skin. The role of individual ceramide classes in the SC structure is still not clear. The ultra-long ω-acylCer (EO-class Cer), which contain 30-34C acyls with linoleic acid ester-linked to ω-hydroxyl, are indispensable in the homeostasis of the skin permeability barrier[3]. They are concerned to be essential for the formation of the long periodicity phase. With the aim to shed more light on the ceramide structure - SC lipid model arrangement relations, we prepared samples with Cer having long (C24) or ultra-long (EO-class Cer) acyl chains for the experiment on DN-2 neutron diffractometer. The samples were prepared in a form of aligned oriented multilayers, whose preparation can often be an art more than a science.

Experiment

We synthesized CerEOS belonging to the EO-class Cer, which is not commercially available, and d-$_{47}$CerNS24 with deuterated acyl chain of 24 carbons. We prepared 4 lipid mixtures Cer/Chol/FFA/CholS so that we kept the molar ratio Cer/FFA = 1:1 and the content of CholS of 5 wt. %. We decreased the Chol molar ratio relative to the physiological levels to Cer/Chol/FFA = 1:0.6:1. This composition was determined suitable in the preliminary X-ray diffraction experiments, because Chol showed very low phase separation and the lipid lamellar structure remained preserved. Further we prepared additional 4 samples with more complex Cer composition for contingency reasons.

The proper molar ratio of EO-class Cer:long acyl Cer (0.3:) was selected by preliminary X-ray diffraction experiments. The EO-class Cer was introduced with the aim to investigate the structural changes it generates in the membrane. The replacement of CerNS24 by d-$_{47}$CerNS24 in the parallel samples enables to detect the arrangement of CerNS24 acyl in the membranes. The neutron diffraction was measured at 100% D_2O at T=32 °C.

Results and discussions

The neutron diffraction patterns measured at the conditions specified below showed first order reflection of the samples with short periodicity phase (~ 5.2 nm) and second order reflection of the samples with long periodicity phase (~ 10.2 nm). These peaks were however of low intensity and suggested large mosaicity of the sample, i.e. low alignment order. During the experiment, the samples were therefore repeatedly annealed at increased temperature (90 °C), hydrated and remeasured with the aim to improve their layer organization. However, the additional heating at the conditions, which we standardly used for the SC model samples homogenization, worsened the resulting quality of the lipid lamellar arrangement. Unfortunately, the low number of obtained reflections does not allow calculating the neutron scattering length density profiles. Mojumdar *et. al.* recently measured neutron diffraction of a similar SC lipid model system at two different detector positions[5]. They used lower temperature close to the melting temperature (~ 70 °C) for the sample equilibration. We suppose, the most probably we need to modify the temperature and duration of the sample equilibration to achieve better lipid organization.

Conclusions

We prepared and measured the proposed SC lipid model samples and the additional SC lipid model samples with more complex Cer composition. The neutron diffraction patterns showed first or second order reflection of the periodically arranged lipid lamellar lattice. More reflections would be needed to calculate the neutron scattering length density profiles. Obtaining the higher number of reflections requires better lipid arrangement with higher fraction of the lipid lamellae stacked parallel. The measured lipid mixtures are preserved and could be again dissolved and used for the sample preparation. Our future experiment will focus on the sample homogeneity and equilibration protocol, which seems to be a key step for the proper lipid arrangement development.

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