On route to stratum corneum - the role of ceramides towards model skin

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The project is a long-term project and aims at contributing data to the physics of skin.

Skin tissue consists of a unique pattern of lipids, mostly ceramides that provide the peculiar combination of trans-tissue permeability with simultaneous stability at high elasticity. We intend to assemble physical properties on selected systems that might reveal some of the peculiarities that are induced into the lipid kingdom by ceramides.

For the first experimental session at Hasylab, our primary goal for the investigation of the role of ceramides in (human) skin was to collect data on the modifications that small amounts of ceramides induce when incorporated into well-known host membranes. Membranes of pure POPC (1-palmitoyl-2-oleoyl--sn-glycero-3-phosphatidylcholine) were chosen as a reference system for this initial diffraction session at A2. This lipid fits, from the point of view of shear chain lengths, roughly to the first asymmetric ceramide selected for incorporation: Cer3 [1] (ceramide3; C22:0 chain, amide linked to phytosphingosine + C15-chain). Experiments were conducted on both the pure systems (POPC and Cer3), and as well on composite systems of equal total lipid amount but varying molar composition, namely 5%, 10%, and 15% (mol/mol) of admixed ceramide. The preparation was conducted such that full mixing of the two components used was achieved by co-solving them in the same solvent. Only after complete evaporation of the solvent, water was added in excess to the dried lipid mixture in order to obtain a lipid content of 20% (wt/wt) on average. This lipid/water content was fixed as precisely as possible for all samples, and the samples were homogenized as good as possible; still, the uniformity may have varied among them, especially as the amount of Cer3 is increased. Quantifiers of host membrane disturbances due to the presence of a ceramide can be the phase state as represented by a structural transition and its temperature dependency, the collaborativity of the transition, and the related structural details as the crystallographic system and the related distances.

At first we tried to explore the effect of the ceramide admixture on the thermotropic lamellar phase state of the reference membrane. POPC has a melting transition (main phase transition) at ~270K. Unfortunately, the current thermosetting at A2 turned out not the reach this temperature: we acquired data only in the range [270K, 353K] but could not stabilize the sample at a constant temperature below 273K. Therefore this part of the experiment must be repeated later. Another choice of host lipid was considered, e.g. DMPC (1,2-di-myristoyl-sn-glycero-3-phosphatidylcholine) or DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine). The first option was rejected because experiments on the system Cer3/DMPC unavoidably would interfere with effects of chain mismatch and would thus shift the focus of the study. DPPC was not available in sufficient amounts.

The results obtained from the diffraction studies are depicted in figure 1.

The spacings and their courses vary among the samples: for pure Cer3 an almost linear increase of the spacing [4.19nm,4.41] is seen for the temperature interval [0°C,80°C]. Cer3 never crossed over its melting temperature (~103°C) into a liquid crystalline phase: most probably the diffraction pattern could be indexed for a solid-like structure. However, the chosen angle range was insufficient for that.
Pure POPC [2] exhibits the expected initial drop of the d-spacing on heating [6.56nm, 6.37nm] for the temperature interval [0°C,20°C[*]]. Two coexisting lamellar phases were observed for the mixed

[*] full temperature range could not be analyzed due to storage failure

systems. The presence of Cer3 caused an increase of d-spacing of the host phase (e.g.: Cer3/POPC=15/85 [6.70nm,6.89nm] throughout the whole temperature interval [0°C,80°C]). The temperature course was as well smoothened. The variation among the three mixtures was insignificant thus hinting towards a saturation of the host phase at low Cer3 contents.

The system was further studied for its thermodynamics by scanning calorimetry and for the morphology of giant vesicles by 2color- confocal microscopy. The results are essentially confirmed: POPC reacts sensitive to the presence of Cer3. Lateral phase separation results in the formation of domains [3] (see figure 2). A paper presenting the more detailed results is in preparation.

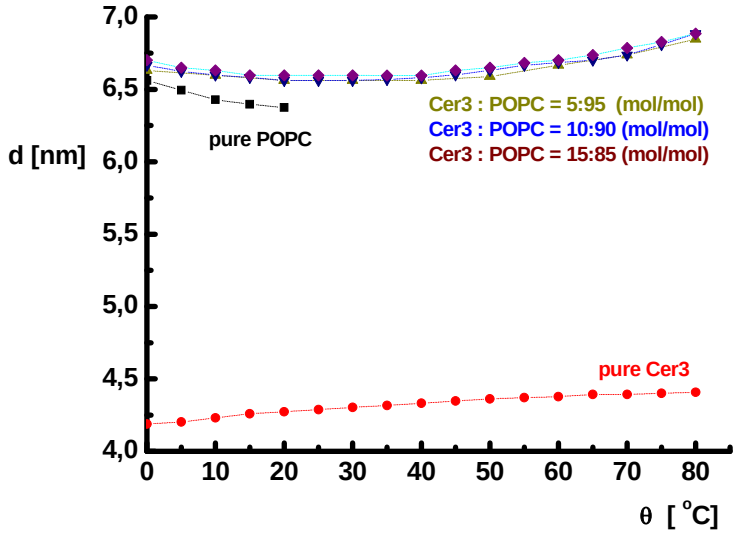

Figure 1: Measured d-spacing as a function of temperature, shown for the fully hydrated pure samples of POPC (black) and CER3 (red), respectively. Mixed samples yield two sequences of (lamellar) reflections. The d-spacing of the POPC rich phase are shown above suggesting a saturation at low Cer3 uptake.

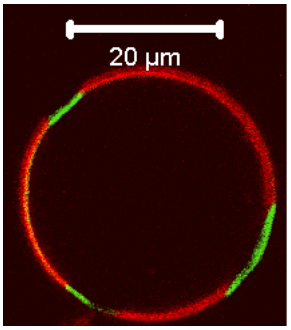

Figure 2: 2color-confocal image of a giant vesicle prepared from a tentative mixture of Cer3:POPC=5:95 (mol/mol). The lipid is labeled by two dyes (Bodipy-PC and DiI-C18, supplied by Invitrogene) that distribute differently depending on their lipid surroundings, thus serving as domain labels. The facetted feature of the green domains suggests the presence of a solid phase.

References

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