Experimental report

13/02/2017

Proposal: 8-02-762 **Council:** 4/2016

Title: Investigating the ultrastructure of an archaeal membrane containing apolar structural lipids

Research area: Biology

This proposal is a new proposal

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Samples: 1,2-di-O-phytanyl-sn-glycero-3-phosphocholine
deuteurated squalane
1,2- di-O-phytanyl-sn-glycerol
squalane

Instrument	Requested days	Allocated days	From	To
D16	7	6	28/11/2016	05/12/2016

Abstract:

The main objective of this project is to test experimentally the validity of a novel membrane model proposed to explain the composition and stability of the membranes of the hyperthemophilic archaea Thermococcus barophilus. This model proposes that apolar lipid molecules populate the midplane of the membrane bilayer, increasing membrane rigidity, impermeability and temperature resistance. We will reconstruct archaeal membranes in presence or absence of apolar lipids and determine the impact of apolar lipids on the membrane ultrastructure and characterize the impact of the intraplanar lipids layer on the specific physico-chemical characteristics of the membrane.

Report on D16 experiment 8-02-762

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Investigating the ultrastructure of an archaeal membrane containing apolar structural lipids

Cell membranes have a fundamental role in the structure and cellular functions and they are adaptable to environmental changes (e.g. temperature, hydration…). Recently, a new model of membrane ultrastructure has been proposed for the membrane of hyperthermophilic archaea. The novel model suggests that the inner plane of the archaeal bilayer membrane is populated by apolar lipids [Cario 2015, Oger 2013], which would extend the stability of the membrane to higher temperatures [Haines 2001]. Neutron diffraction scattering has already been used to demonstrate the validity of this model for bacteria [Hauß 2002].

Project:

Our original project was to study the structural characteristics of oriented membranes reconstructed from a mixture of archaeal polar lipids (100% monopolar, 100% bipolar and 50% of each) and apolar lipids (in presence or not of squalane or d62-squalane) to identify how the apolar lipids are situated in the membrane. Monopolar archaeal lipids and squalane are available as synthetic lipids. We used 1,2-diphytanoyl- *sn*-glycero-3-phosphocholine (DPhPC) and 1,2-diphytanoyl- *sn*-glycero-3-phosphoethanolamine (DPhPE) in a mixture of 90:10 mol %. Bipolar lipids are not commercially available, and had to be purified from an hyperthermophilic archaeaon. Unfortunately, bipolar archaeal lipids could be extracted from cells of the hyperthermophilic archaea *Thermococcus sibiricus* in sufficient quantities in time for the experiment.Therefore, we studied the structural characteristics of oriented membranes from DPhPC_DPhPE in the presence or not of squalane (or d62-squalane). In addition, we studied oriented membranes made of the natural monopolar lipids. For both membranes, we used the BerILL hydration chambers on D16. Taking advantage of the BerILL chambers, we could do a scan in temperature (from 25°C to 70°C in steps of 15°C) and another scan in humidity (50%, 80%, 90%, 95%, 98% and 100% of humidity) for both types (natural and synthetic) oriented membranes without squalane. For all the scans, we also recorded the equilibrium process to be sure that it was reached and to observe possible variations between the points.

The results from synthetic archaeal lipids allowed us to confirm the position of apolar lipids inside the bilayer. As presented in the model and previously demonstrated for bacterial lipids, we can now affirm that apolar lipids are situated in the inner plane of the bilayer membrane formed by archaeal lipids (see figure 1). When squalane is present, the lamellar repeat spacing of the lipid bilayer increases by 3.5 Å due to an increase of the hydrophobic region.

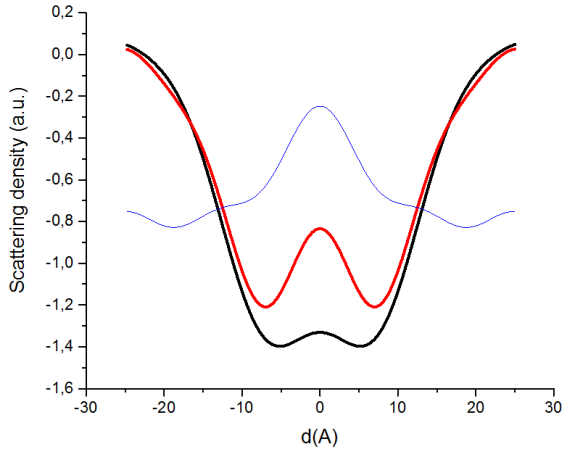

Figure 1. Scattering density function of the DPhPC:DPhPE (90:10 mol%) membrane containing protonated squalane (black line) or deuterated squalane (red line), 20% D_2O contrast. The blue line represents the difference between both scattering densities, showing the localization of the squalane in the bilayer.

High humidity also causes an increase of the lamellar repeat spacing due to an increased water layer thickness. The equilibrium scans of DPhPC_DPhPE between 80% and 90% humidity also show a phase transition. The two lamellar phases coexist at 90% of humidity (see figure 2). Looking at the scattering length density function of DPhPC_DPhPE, we can observe a rearrangement of the hydrocarbon chains in function of the humidity. All this indicates that the organization of the hydrocarbon chains is very sensitive to their level of hydration.

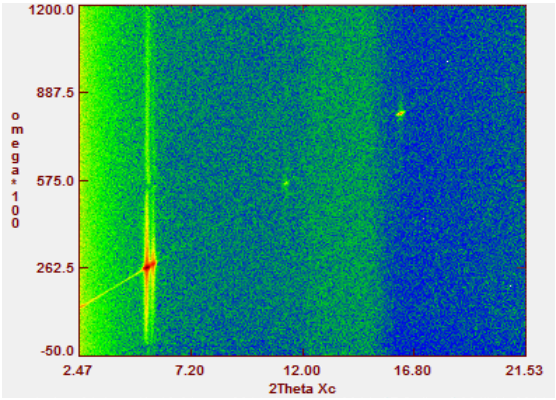

Figure 2. Diffraction pattern of DPhPC:DPhPE (90:10 mol%) membrane at 25°C and 90% humidity. There is a coexistence of two lamellar phases.

The scan in temperature of DPhPC_DPhPE shows a possible reduction of the lipid bilayer organization as the temperature is increased, with a critical point at 55°C. However, we will complete these first results performing permeability studies for these lipids.

The natural lipids from *T.sibiricus* are less ordered than lipids from DPhPC_DPhPE and in all cases but one, the diffraciton pattern only shows a weak first order peak. A second order of diffraction is only visible when squalane is incorporated in the membrane (see figure 3). In this case, we observe the coexistence of two lamellar phases, which could be caused by the presence of squalane, suggesting that squalane increases the ordering of the membrane.

Our observations suggest that squalane is not saturating the membranes composed of natural archaeal lipids. In further experiments, it would be interesting to measure the effect of the proportion of squalane relative to other membrane lipids to see how it affects the stability of these two phases and consequently how it effects the bilayer structure and organization.

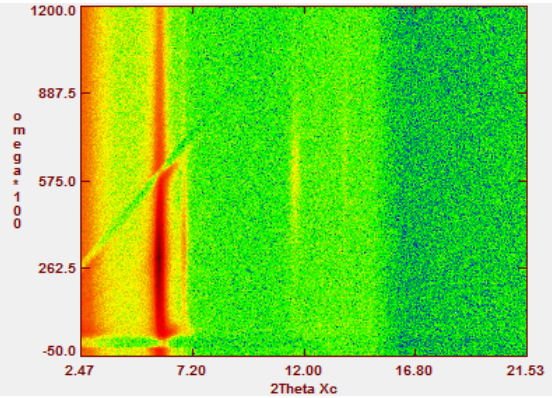

Figure 3. Diffraction pattern of lipids from T.sibiricus containing 10 mol% of squalane. Two different phases coexist.

The humidity scans for the lipids from *T.sibiricus* indicate that these lipids are difficult to rehydrate, although higher levels of hydration show an increase in the order of the bilayers.

Last, scans in temperature for these lipids could not be obtained because of the loss of signal when the temperature was increased. THis loss is essentially caused by a huge dehydration of the sample. Nevertheless, we observed an abrupt phase transition when the humidity was increased from 50% to 98% at 55°C.

References

Cario, A., Grossi, V., Schaeffer, P., and Oger, P. (2015). Front Microbiol **6**.doi: 10.3389/fmicb.2015.011152
Haines, T.H. (2001). Prog Lipid Res **40**: 299-324.
Hauß, T., et al. (2002). Biochim Biophys Acta **1556**: 149-154.
Oger, P., and Cario, A. (2013). Biophys Chem **15**: 42-56.