Experimental report

18/11/2020

Proposal: 8-02-884 **Council:** 10/2019

Title: Relative contribution of the polarmoieties of archaeal ether lipids on membrane parameters

Research area: Biology

This proposal is a new proposal

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Samples: DoPhPC + DoPhPE
DoPhPG
DoPhPS

Instrument	Requested days	Allocated days	From	To
D16	10	7	11/09/2020	18/09/2020

Abstract:

We have recently proposed a novel membrane architecture model to explain the stability of lipid bilayers of archaeal cells at high temperatures (> 70°C) and high pressures (400 bar). In this architecture, the increase in membrane stability/rigidity is due to the presence, in the midplane of the bilayer, of apolar hydrocarbons. Our previous experiments (see ILL report 8-02-762 and 8-02-809) have demonstrated that the apolar molecules are located inside the membrane in the midplane of the bilayer. Using different approaches (FTIR, SAXS, DSC), we have demonstrated the impact of the presence of squalane inside the membrane on the physicochemical properties of the bilayer, and especially the predicted increase in stability/rigidity. Membrane parameters depend only partially on core lipid composition, but depend also largely on the polar headgroup properties. In this experiment, we now want to measure the impact of 4 different polar headgroups, more closely related to the natural polar heagroups of Archaea, on membrane properties, in order to evaluate the relative contribution of the polar and apolar moities in membrane physical parameters.

Report on Experiment # 8-02-884: Relative contribution of the polar moieties of archaeal ether lipids on membrane parameters

Introduction: Life has been found under some of the most extreme (temperature, pressure and pH) conditions on Earth. These extreme conditions can have detrimental effects on cells, inducing protein or membrane malfunctions. Membranes are especially sensitive and small variations temperature can induce important functional defects. Archaea have membranes consisting of phospholipids that highly differ from usual bacterial lipids and which are also more stable at high temperature (HT). Archaeal lipids consist of *sn*-glycerol-1-phosphate backbone, ether linkages, and phytanyl hydrocarbon chains while most bacterial lipids include *sn*-glycerol-3-phosphate backbone, ester linkages, and straight acyl chains [1]. The lipid compositions of cell membranes are regulated in response to environmental conditions in a process called homeoviscous adaptation. Adaptions in archaeal membranes to extreme conditions include the synthesis of bipolar ether lipids capable of forming monolayer membranes and changes in the composition of the polar headgroups. In bacteria, the modulation of acyl chain length is a well-known mechanism of membrane adaptation. In most Archaea, the lipid chain lengths are invariable; fixed at 20 carbons for the monopolar lipids and 40 carbons for the membrane-spanning, monolayer forming lipids. Thus, we expect the nature of polar headgroups to play an even more significant role in the membrane adaptation of archaea. Previous experiments have relied on "neutral" lipids such as DoPhPC which are commercially available [2,3]. However, the phosphocholine (PC) headgroup is not found in abundance in archaea. As part of the ANR archaeaomembranes project have synthesized archaeal like membranes with charged polar head groups commonly found in archaea: phosphoinositol (PI), phosphoserine (PS) and phosphoglycerol (PG) [4]. In this work, we looked at the behavior of lipid multilayers containing charged polar head groups using neutron diffraction as a function of temperature.

Results: Neutron diffraction was performed using several different D_2O contrasts (8, 50, and 100% D_2O). We compared membranes composed of the neutral lipid DoPhPC and membranes composed of a 1:1 mixture of DoPhPC with either DoPhPI, DoPhPS, or DoPhPG. Diffractograms of membranes composed of DoPhPC or DoPhPC:DoPhPI mixed in a 1:1 ratio (PI:PC) at 25 °C are shown in Figure 1.

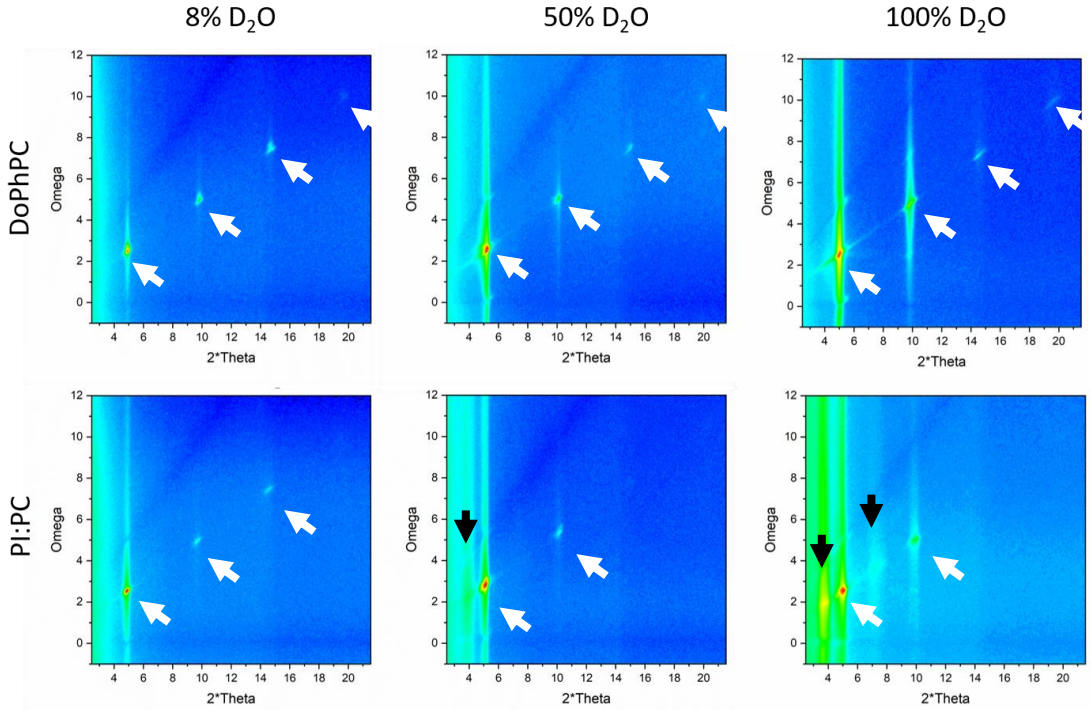

Figure 1: Neutron diffractograms of a DoPhPC membrane (top) or a 1:1 mixture of DoPhPI:DoPhPC (bottom) at different D_2O contrasts: 8% D2O (left), 50% D2O (center) or 100% D2O (right). A single lamellar phase is seen in the DoPhPC membrane (white arrows). A similar phase is seen in the DoPhPI:DoPhPC membane (white arrows) and an additional phase (black arrows) is seen in the PI:PC membrane at 50 and 100% D_2O contrast.

At all contrasts, a single lamellar phase was seen for the DoPhPC membrane. The PI:PC membrane however showed a second lamellar phase, only visible at high D_2O contrast. The lamellar repeat spacing (d-spacing) of the phase indicated with white arrows was ~ 54 Å and the d-spacing of the phase indicated with black arrows was ~ 72 Å at 25 °C.

In addition to DoPhPC and PI:PC, we looked at membranes composed of a 1:1 mixture of DoPhPS:DoPhPC (PS:PC) and DoPhPG:DoPhPC (PG:PC). Similar to the larger phase seen in the PI:PC membrane, diffraction was only seen at high D_2O contrasts (50, 100%). No diffraction was detected at a contrast of 8% D_2O. All four membrane compositions were studied as the temperature was increased from 25°C to 85°C. For each membrane system we see at least two orders of diffraction allowing for accurate calculation of the membrane d-spacing. The membrane composition significantly affected the membrane response to increasing temperature. The location of the Bragg peaks shifted in response to temperature, indication a change in the membrane d-spacing. The d-spacing values calculated for each of the four membrane compositions are shown as a function of temperature (Figure 2). The DoPhPC membrane d-spacing increased at a rate of +0.049 ± 0.004 Å/°C as the temperature increased. The PI:PC membrane had two lamellar phases which behaved differently in response to pressure. Phase 1 (~72 Å at 25°C) had a d-spacing which decreased at a rate of -0.182 ± 0.006 Å/°C. Phase 2 (~54 Å at 25°C) increased at a rate of +0.056 ± 0.016 Å/°C. The PS:PC and PG:PC membranes also showed a decrease in d-spacing with increasing temperature at rates of -0.087 ± 0.009 Å/°C and -0.099 ± 0.009 Å/°C respectively.

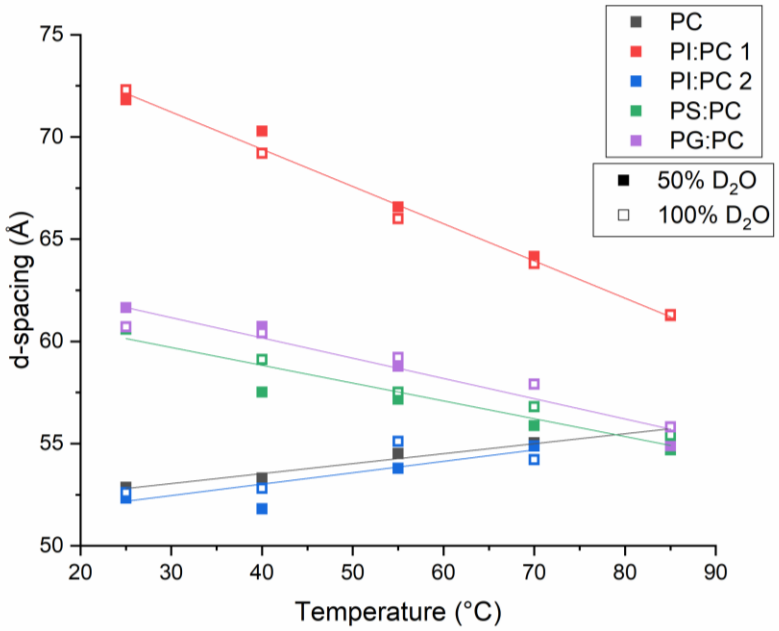

Figure 3: Change in membrane d-spacing (Å) as a function of temperature (°C). The d-spacing of DoPhPC (black), DoPhPI:DoPhPC phase 1 (red) and phase 2 (blue), DoPhPS:DoPhPC (green) and DoPhPG:DoPhPC (purple) determined from diffraction data at either 50% D_2O (solid symbols) or 100% D_2O (open symbols).

In membrane phases which were visible at 8% D_2O contrast (DoPhPC, PI:PC Phase 2) the phases showed an increase in d-spacing as the temperature increased of ~3 Å from 25°C to 85°C. This is most likely due to an increase in the associated water layer as seen previously in a DoPhPC:DoPhPE (9:1) membrane [5]. In phases not visible at 8% D_2O contrast (PI:PC Phase 1, PS:PC, PG:PC), the d-spacing decreased with temperature. The PS:PC and PG:PC membranes decreased ~ 5 Å from 25°C to 85°C. The largest change was seen in Phase 1 of the PI:PC membrane which decreased ~10 Å from 25°C to 85°C. This is most likely due to a decrease in the associated water layer.

References: [1] Gambacorta, A. et al. *Syst. Appl. Microbiol.*, 1993, **16**, 518–527 ; [2] Salvador-Castell, M. et al. (2020) *BBA – Biomembranes* **1862** ; [3] LoRicco, J. et al. (2020) Frontiers in Chemistry ; [4] Tourte, M. et al. (2020) *Biomolecules* **10**