Experimental report

18/05/2021

Proposal: 8-02-919 **Council:** 4/2020

Title: Impact of polar headgroup moities on archaeal membrane parameters

Research area: Biology

This proposal is a new proposal

Main proposer: Josephine LORICCO

Experimental team: Josephine LORICCO
Judith PETERS
Philippe OGER
Agathe NIDRICHE

Local contacts: Bruno DEME

Samples: DoPhPS
DoPhPI
DoPhPC

Instrument	Requested days	Allocated days	From	To
D16	10	10	12/02/2021	22/02/2021

Abstract:

We have proposed a novel membrane architecture to explain the stability of lipid bilayers of archaeal cells at high temperatures and pressures. In this architecture, the increase in membrane stability/rigidity is due to the presence in the midplane of the bilayer of apolar molecules. In previous experiments (8-02-762/8-02-809) using a mixture of archaeal lipids with phospho-choline and -ethanolamine headgroups, we have demonstrated that the apolar molecule is located inside the membrane. Using different approaches (FTIR, SAXS, DSC), we further demonstrated the predicted increase in stability/rigidity and impact on permeabilty. Membrane parameters depend largely on polar headgroup properties. In the current experiment, we now want to explore the differential behavior of the membrane as a function of the nature of the polar headgroup moity using 4 novel synthetic archaeal lipids made available through the ANR ArchaeoMembrane program. These lipids have chemical structures more closely related to natural lipids of Archaea. Using these lipids we will be able to evaluate the relative contribution of the polar and apolar moities on membrane physical parameters in the archaeal bilayer.

Experiment# 8-02-919: Impact of polar headgroup moieties on archaeal membrane parameters

Introduction: Archaeal membrane phospholipids differ from those of bacteria/eukaryotes in three major ways. (1) Branched phytanyl chains rather than straight acyl chains, (2) Ether rather than ester linkages and (3) G1P rather than G3P backbone stereochemistry [1]. Model lipids systems most often rely on lipids possessing a phosphocholine (PC) headgroup which are known to be commonly found in bacteria/eukaryotes. However, archaea (as well as bacteria/eukaryotes) have membrane lipids with many different polar headgroups [2-4]. Additionally, PC is not a common archaeal lipid headgroup. To create a better model of archaeal lipids it is important to consider other polar headgroups. This study probes the characteristics of membranes made of archaeal-like lipids, containing different polar headgroups. All the phospholipids in this study are archaeol based with identical phytanyl chains linked to the glycerol backbone via ether bonds (DoPhPX where X is the identity of the polar headgroup). Headgroups include choline, L-serine, glycerol, and *myo*-inositol which can all be synthesized in archaea. The backbone stereochemistry are as follows: DoPhPC-(R), DoPhPS-(R, S, or racemic), DoPhPG-(racemic), and DoPhPI-(racemic); where R is the natural bacterial configuration (G3P) and S is the natural archaeal configuration (G1P). This experiment probes the effect two different important lipid characteristics: (1) the role of the polar headgroup, and (2) the importance of the backbone stereochemistry. As many archaea are capable of at high temperature, measurements were made from 25 - 85°C, to see if any of the polar headgroups are advantageous in these conditions.

Sample Preparation & Experimental Setup: 3mg of lipid was deposited on an ultraclean silicon wafer in organic solvent. The solvent was evaporated, and residual solvent was removed by placing the wafer under vacuum overnight. Lipids were sealed in a flat aluminum cell and hydrated by D_2O/H_2O water vapor at 40°C for at least 12 hours. A single detector position (Γ = 12.2°) was used and omega scans performed from -1 > Ω > 12° (0.05° steps). The $\lambda_{neutron}$ = 4.466Å.

Results: Neutron diffraction was performed on different archaeol-based lipids containing different polar headgroups. Lipids containing a PG or PS headgroup diffracted poorly showing only first order diffraction peaks. The lipids containing the PI headgroup showed a second order of diffraction in some cases. Only the phosphocholine (PC) headgroup led to the appearance of more than two orders of diffraction. It should be noted that while the PC headgroup has a net neutral charge; PS, PG, and PI all carry a net negative charge. The membrane d-spacing was calculated from the first order diffraction peaks (Table 1). This method was used for all lipids, even those showing more orders of diffraction to keep the method of analysis consistent. This method typically has errors of between 1 and 1.5 Å. While using a single peak is not ideal, it is a good estimate of the membrane d-spacing, and can be used to track changes in membrane d-spacing as a function of temperature. DoPhPC and DoPhPSrac showed increases in d-spacing as a function of temperature. DoPhPS-(S), DoPhPG, and DoPhPI decreased with temperature, and DoPhPS-(R) remained fairly constant. Both DoPhPG and DoPhPI had a second lamellar phases present at low temperature. These phases disappeared at high temperature.

Table 1: d-spacing (Å) estimated from first order diffraction peaks. Errors are approximately 1 Å.

	25°C	40°C	55°C	70°C	85°C
DoPhPC	52.4	52.7	52.5	53.4	55.5
DoPhPS-(R)	53.5	53.7	54.4	54.3	53.9
DoPhPSrac	48.1	47.4	47.7	48.7	49.8
DoPhPS-(S)	46.6	45.4	43.7	41.3	39.8
DoPhPG	47.4*	45.8*	44.2	42.5	40.9
DoPhPI #1	69.1	64.7	64.0	62.2	59.8
DoPhPI #2	48.7	47.8	--	--	--

* indicates the presence of another minor phase that could not be accurately fit.

Mixtures of two lipids (with the exception of those mixed with DoPhPC) did not show a marked improvement in the diffraction, typically exhibiting only first order diffraction peaks. The d-spacing of these mixtures are shown in Table 2. Interestingly, lipids mixed with DoPhPC often showed multiple lamellar phases, and larger d-spacing values than seen with individual lipids.

Table 2: d-spacing (Å) estimated from first order diffraction peaks. Errors are approximately 1 Å

	25°C	40°C	55°C	70°C	85°C
PS-(R):PC #1	59.2	60.3	61.0	61.1	60.2
PS-(R):PC #2	54.1	55.0	56.0*		
PS-(S):PC #1	53.1	56.4	58.6	59.9	58.2
PS-(S):PC #2	49.9	51.3	51.6*		
PI:PC #1	78.3	70.5	66.9	63.8	61.1
PI:PC (PC-like)	52.1	52.5			
PI:PSrac	64.3	62.2	59.7	57.4	55.2
PI:PG	52.9*	51.1	49.2**	47.3**	45.6**
PSrac:PG	53.0	51.4	49.9	48.1	46.5
PI:PSrac:PG:PC	63.2*	60.6*	58.3*	56.3*	54.2

* or ** indicate the presence of another minor phase that could not be accurately fit.

Mixtures of PS-(R):PC, PS-(S):PC and PI:PC all showed multiple lamellar phases with ≥ 2 orders of diffraction. In the PI:PC membrane at 25°C not only do we see the presence of two coexisting lamellar phases, but also see the appearance of peaks in the q_x direction (Figure 1) indicating the presence of lateral organization within the membrane. One of the phases (denoted with *) gave four orders of diffraction. The d-spacing of this phase was found to be ~52 Å which is very similar to the d-spacing of a pure DoPhPC membrane. A neutron scattering length density (NSLD) plot was generated for both PI:PC* and pure DoPhPC. The two NSLD plots were found to overlap almost perfectly, suggesting that the PI:PC* phase is composed of entirely DoPhPC. The other PI:PC phase has a d-spacing of ~78 Å, which is much larger than the larger phase seen for DoPhPI alone (~ 69 Å) suggesting this phase is PI-rich but still a mixture of DoPhPI and DoPhPC. It is interesting to note that in membrane phases containing mixtures of charged lipids and DoPhPC, the mixture always has a significantly larger d-spacing than the charged lipids or DoPhPC alone. This was also seen with our previous experiment that looked at mixtures of PSrac:PC and PG:PC (Exp. #8-02-884).

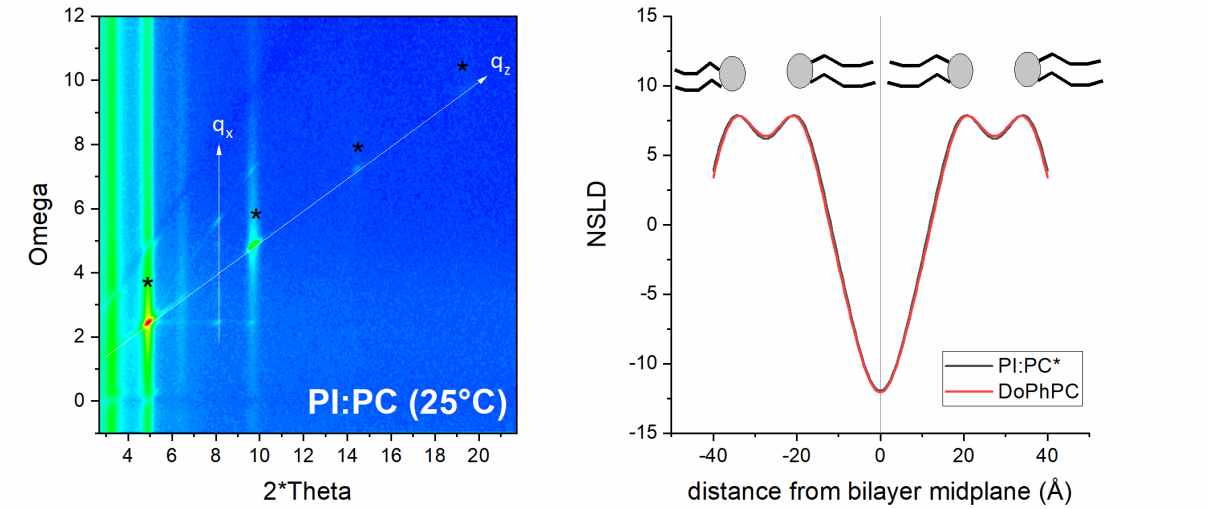

*Figure 1: Lateral organization in DoPhPC:DoPhPI (1:1) mixture at 25°C. (Left) 2D diffractogram showing appearance of Bragg peaks in the q_x direction in addition to the q_z direction. Two lamellar phases are detected in the data. One of the phases (denoted with *) has four orders of diffraction which is sufficient to produce a NSLD plot. (Right) NSLD plot comparing PI:PC phase* (black) with NSLD of pure DoPhPC (red).*

References: [1] Gambacorta et al. (1993) *Syst. Appl. Microbiol.*; [2] Koga et al. (1993) *Syst. Appl. Microbiol.*; [3] Sprott (2011) "eLS"; [4] Tourte et al. (2020) *Biomolecules*