Experimental report

24/08/2021

Proposal: 8-02-951 **Council:** 10/2020

Title: The influence of charged polar headgroups on the structure and stability of archaeal membranes at high pressure and temperatures

Research area: Biology

This proposal is a continuation of 8-02-852

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Samples: DoPhPG
DoPhPS
DoPhPI
DoPhPC

Instrument	Requested days	Allocated days	From	To
D16	8	7	01/07/2021	08/07/2021

Abstract:

Archaeal lipid membranes are able to remain functional at high temperatures (>70°C) and high pressures (>400 bar) in part due to their unique lipids which contain ether rather than ester linkages and their branched isoprenoid hydrocarbon chains. Archaea are known to have lipids with diverse polar headgroups and that the lipid composition of the membrane varies with pressure and temperature. Here we propose to study what role the lipid polar heads play in adaption of archaea to high temperature (HT) and high hydrostatic pressure (HHP). Using neutron diffraction we can probe how membrane structural parameters change as a function of HT and HHP and compare how these parameters differ between archaeal lipids containing several common polar headgroups found in archaea.

Experimental Report #8-02-951: The influence of charged polar headgroups on the structure and stability of archaeal membranes at high pressure and temperature

Introduction: Archaeal lipid membranes are able to remain functional at high temperatures (>70°C) and high pressures (>400 bar) in part due to their unique lipids which contain ether rather than ester linkages and their branched isoprenoid hydrocarbon chains. Archaea are known to have lipids with diverse polar headgroups and that the lipid composition of the membrane varies with pressure and temperature. Here we studied four archaeal-like lipids with different polar headgroups: phosphocholine (PC), phosphoserine (PS), phosphoglycerol (PG) and phosphoinositol (PI) to better understand the role that the polar headgroup plays in the adaption of archaea to high temperature (HT) and high hydrostatic pressure (HHP).

Results: DoPhPC shows little to no change in d-spacing as a function of pressure for T ≤ 55°C (at least within the error of 0.5 -1.0 Å). At 70°C, a slight increase in d-spacing can be seen with increasing pressure (~1 Å/kbar). As shown previously, we also see a slight increase in the DoPhPC membrane d-spacing with increasing temperature (~0.04 Å/°C). DoPhPS also shows slight increases in d-spacing with increasing pressure which is most noticeable at low temperatures ≤40°C (~2 Å/kbar), close to the error at 55°C (~1 Å/kbar), and no significant change at 70°C. Unlike DoPhPC membranes which experience and increase in d-spacing as a function of temperature, the DoPhPS membrane decrease in d-spacing with increasing temperature (~0.16 Å/°C). DoPhPG shows clear increases in d-spacing with pressure with the exception of the measurement taken at 25°C. This is likely due to the broad diffraction peaks at this temperature, likely indicating multiple phases with similar spacing. If this temperature is ignored due to the complexity of the multiphase system we see increasing in d-spacing on the order of ~1.4 - 3.6 Å/kbar, and a decrease in d-spacing with temperature of ~ 0.15 Å/°C which is similar to DoPhPS.

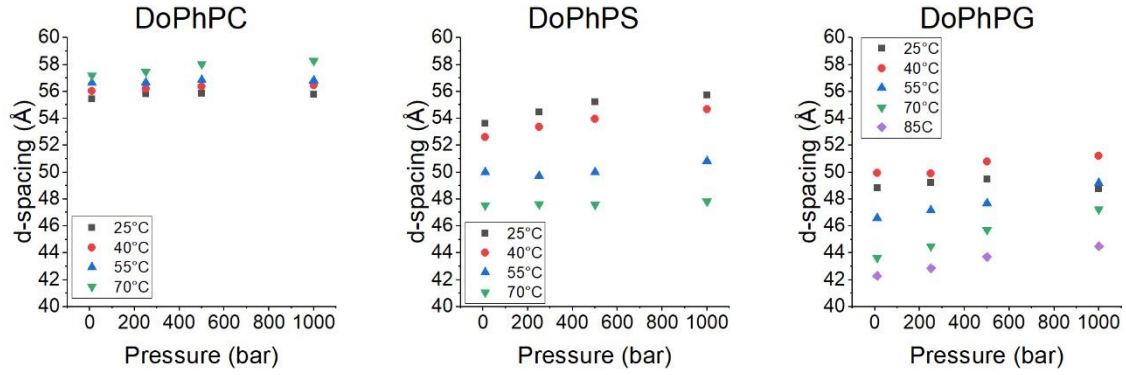

Figure 1: Change in d-spacing with pressure of DoPhPC (left), DoPhPS (center) and DoPhPG (right). Errors are approximately 0.5 -1 Å.

The DoPhPI membrane interestingly consists of two phases at low temperature/high pressure. Phase 1 with a much larger d-spacing (60.9 – 78.0 Å), and Phase 2 with a smaller d-spacing (48.0 - 49.3Å). While Phase 1 was present at all T/P tested, Phase 2 gives a high intensity peak at low temperature which decreases rapidly in intensity and finally disappears at elevated temperatures. The intensity of the peaks for Phase 2 decreased linearly with temperature. A global fit of Peak Intensity vs Temperature for each pressure (slope was fit globally) was used to determine the temperature at which Phase 2 was seen to disappear at each pressure. The resulting phase diagram can be seen in Figure 2. While Phase 2 is destabilized by temperature, pressure stabilizes Phase 2.

Phase 1 of DoPhPI shows the largest increases in d-spacing with pressure (~4-8 Å/kbar) among all the lipids tested (Figure 2). The largest changes were seen in the measurements made at lower temperatures. Similar to DoPhPS and DoPhPI, the d-spacing of Phase 1 decreases with temperature (~0.16 – 0.21 Å/°C).

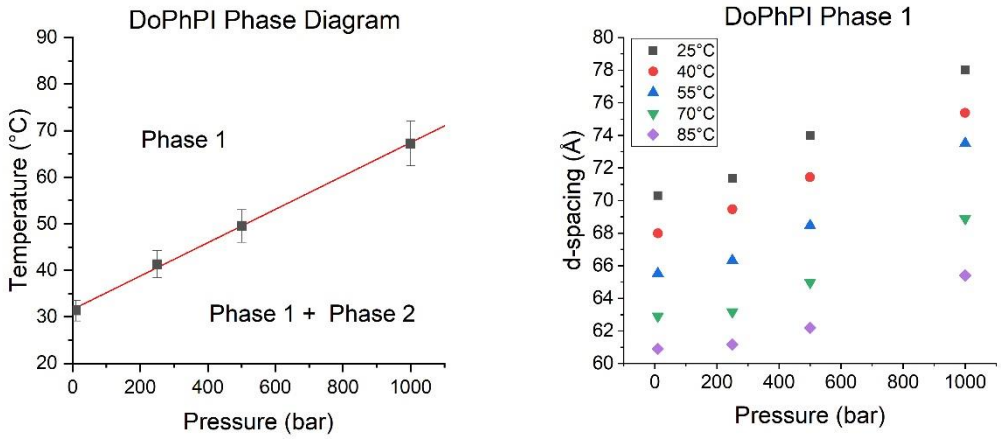

Figure 2: (Left) Phase Diagram of DoPhPI. (Right) Change in d-spacing of Phase 1 as a function of pressure. Errors are approximately 0.5 -1 Å.

Discussion: Striking differences can be seen between the membranes with different polar headgroups in response to both pressure and temperature. We have previously examined the behavior of DoPhPC, DoPhPS, DoPhPG and DoPhPI with temperature in flat aluminum cells (Exp. #8-02-884 & #8-02-919) at ambient pressure. Similar trends were seen in this study at 10-1000 bar. The overall magnitude for d-spacing values of the DoPhPC membrane were higher (~3Å) in this study than seen previously but the trend with temperature was found to be very similar. This is likely a result of the different sample environment, and the application of pressure transmitted using fluorinert. The absolute magnitude of the d-spacing for DoPhPG and DoPhPI were also slightly different from the measurements made in the flat aluminum cells (~1-2Å), but the trends with temperature were almost identical.

The DoPhPS was the only membrane to behave noticeably differently with temperature when in the HP cell exhibiting a decrease in d-spacing with temperature rather than a slight increase as seen previously in Experiment #8-02-919. It should also be noted that d-spacing values presented here were estimated from the first order diffraction peaks as there was often only one peak present – particularly under high pressure and temperature conditions. The errors for these estimates are greater than what would be determined from multiple diffraction peaks.

While DoPhPC increased in d-spacing with increasing temperature, the other three lipids (DoPhPS, DoPhPG and DoPhPI) all exhibit a decrease in d-spacing with increasing temperature. The lipids all contain headgroups with a net negative charge which could contribute to the difference in behavior as a function of temperature. In this study for the first time we looked at the behavior of these diphytanyl lipids in response to pressures up to 1kbar. The DoPhPC, DoPhPS and DoPhPG lipids showed no change to slight increases in d-spacing as a function of pressure. The increases seen in d-spacing with pressure could come from either (1) an increase in the thickness of the water layer or (2) and increase in the thickness of the membrane resulting from compaction of the lipid tails. Unfortunately, the contribution of the water thickness and membrane thickness to the d-spacing could not be determined from our data.

DoPhPI showed the most interesting behavior, with two distinct phases being present under many of the conditions tested. Phase 1 was present at all T/P conditions and showed the largest pressure-dependence of all of the membranes tested. Phase 2 was only present at low-T / high-P conditions. This phase also appeared to show slight increases in d-spacing with increasing pressure but due to a limited number of data points the trends could not be determined accurately.