Experimental report

20/07/2022

Proposal: **8-02-973** **Council:** 4/2021

Title: Effect of apolar lipids on archaeal membranes containing charged headgroups

Research area: Biology

This proposal is a new proposal

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Samples: squalane
DoPhPG
DoPhPS
DoPhPC

Instrument	Requested days	Allocated days	From	To
D16	8	5	26/08/2021	31/08/2021

Abstract:

Many archaea are known to live under high temperature conditions (>85C) and therefore must have specially adapted membranes in order to survive under these conditions. One adaptation strategy found in archaea is the production of monolayer forming tetraether lipids, however not all archaea living at high temperature produce these lipids. Therefore other mechanisms of adaptation must exist. Recently it has been proposed that the presence of apolar lipids, such as squalane, sit that the midplane of the membrane bilayer and provide an alternative mechanism for adaptation to high temperature. In this work we hope study the structure of a model archaeal membranes containing phosphoserine (PS) and phosphoglycerol (PG) headgroups which are commonly found in archaea as a function of temperature. Preliminary results demonstrate that the PS and PG lipids have a lesser ability to self-organize than phosphocholine (PC) containing lipids and that apolar lipids may be required in this lipid system to obtain stable and structured membrane bilayers.

Exp. # 8-02-973: Effect of apolar lipids on archaeal membranes containing charged headgroups

Introduction: Many archaea are known to live under high temperature conditions (>85°C) and therefore must have specially adapted membranes in order to survive under these conditions. One adaptation strategy found in archaea is the production of monolayer forming tetraether lipids, however not all archaea living at high temperature produce these lipids. Therefore, other mechanisms of adaptation must exist. Recently it has been proposed that the presence of apolar lipids, such as squalane, sit that the midplane of the membrane bilayer and provide an alternative mechanism for adaptation to high temperature. In this experiment, we probed the structure of archaeal-like lipids which form lipid bilayers in the presence of 5 mol% squalane and compare to the structure of bilayers lacking squalane. In this study we formed membranes from diphytanyl lipids containing charged polar headgroups, phosphoserine (DoPhPS), phosphoglycerol (DoPhPG), and phosphoinositol (DoPhPI) in the presence of squalane, and studied these membranes as a function of temperature.

Archaeal lipids have hydrocarbon chains which are linked to the glycerol backbone via ether linkages, unlike typically bacterial lipids which have ester linkages. Ether bonds are thought to enhance membrane stability at high temperatures. This is supported by the finding that some bacteria living under high temperature conditions can also produce ether lipids. Therefore, we also measured two ester-linked diphytanoyl lipids, DPhPC and DPhPC, as a function of temperature in order to probe the role of the ether bond on the behavior of phytanyl-chain lipids.

Results: Diffraction of membranes containing DoPhPS, DoPhPG, DoPhPI and squalane

We were able to form lipid multistacks with all of our lipid + squalane membranes that gave at least one order of diffraction allowing us to estimate the membrane d-spacing. As seen previously in membranes lacking squalane, DoPhPG + sq and DoPhPI + sq membranes had two coexisting phases. The presence of squalane in the bilayer did not improve the ordering of the lipid stacks or improve the amount of diffraction seen for the archaeal-like lipids used in this study. Example diffractograms can be found in Figure 1, which show the relatively poor signal to noise ratio for these membranes.

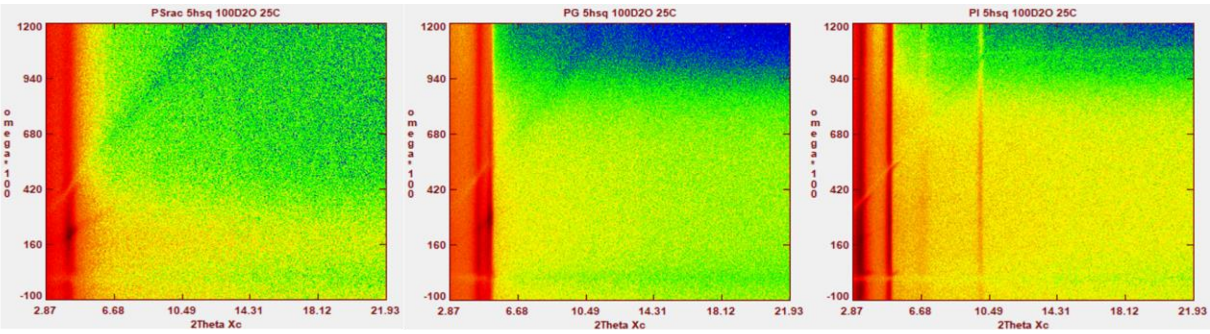

Figure 1: 2D diffractograms of DoPhPS + sq (left), DoPhPG + sq (center), and DoPhPI + sq (right).

The membrane d-spacing were estimated based on the first order diffraction peaks for the membranes + squalane. This data was then compared to data from Exp. # 8-02-884 and #8-02-919 for the same membranes without squalane. The d-spacing values at 25°C are shown in Figure 2.

Differences were seen in the membrane d-spacing for membranes with and without squalane. The largest differences were seen in the membrane containing DoPhPS (PSrac). The addition of squalane to either DoPhPS or a 1:1 mixture of DoPhPS and DoPhPC (PS:PC) lead to membranes with a much larger d-spacing (> 10Å). A larger d-spacing was also seen for membranes composed of DoPhPG, a 1:1 mixture of DoPhPG and DoPhPC (PG:PC), and DoPhPI in the presence of squalane. The only membrane system without a significant change in d-spacing between the membrane with and without squalane was PI:PC (1:1). Small increases in d-spacing could be the results of an increase in the bilayer thickness due to the presence of squalane at the midplane of the bilayer. Larger changes likely indicate a change in the hydration of the membrane.

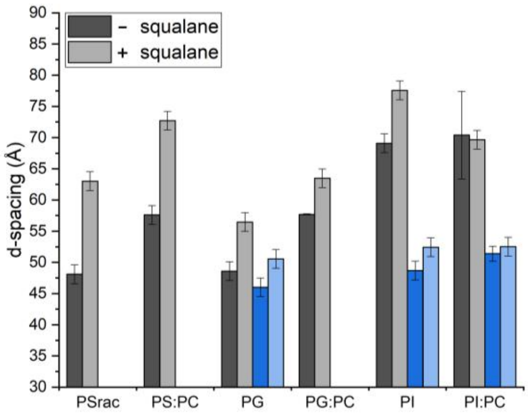

Figure 2: Comparison of membrane d-spacing values from membranes at 25°C in the presence and absence of squalane. The identity of the polar headgroup(s) is shown on the x-axis. In some membranes phase coexistence was seen. The first membrane phase is show in black (-sq) / gray (+sq) and the second membrane phase is shown in blue (-sq) / light blue (+sq).

Diffraction was measured for each of these lipid systems as a function of temperature. Upon completion of the analysis, the behavior of the different membranes will be compared with temperature to further explore the role of squalane as a membrane regulatory. The poor diffraction of these lipids will only allow for limited conclusions to be draw and the additional complementary experiments will need to be performed with these lipid systems.

Results: Diffraction of ester-linked, phytanyl chained lipids with temperature

During this beamtime, we were also able to look at ester-analogs of archaeal-like lipids as a function of temperature (DPhPC and DPhPG). The presence of ether rather than ester linkages is another possible adaptation mechanism to high temperature. We found that the ester-linked lipids swelled dramatically with temperature unlike the ether-linked lipids which studied in Exp. # 8-02-884 and #8-02-919. It was also interesting to note that the PG-ester linked lipid gave 3-4 orders of diffraction, unlike the related PG-ether which only gave one diffraction peak.

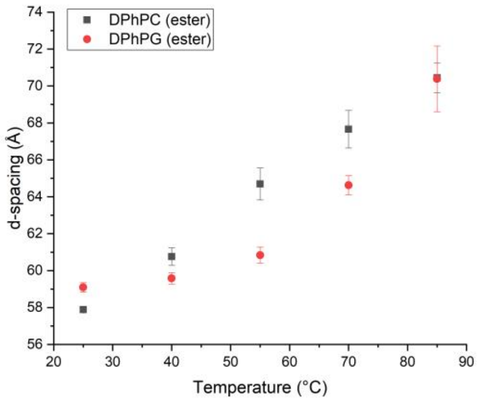

Figure 3: Swelling of ester-linked lipid membranes as a function of temperature.

This data combined with additional diffraction data measured on these ester-linked lipids during Exp. # 9-13-1034 and # 8-02-952 will help to determine the structural differences in membranes formed with ether and ester-linked lipids, and help elucidate a physical mechanism by which ether linkages promote temperature stability.