Experimental report

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Proposal: 9-13-1034 **Council:** 4/2021

Title: Role of ester versus ether bonds in the hydration and temperature stability of branched chain lipids

Research area: Biology

This proposal is a new proposal

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Samples: DPhPC
DphPS
DPhPE

Instrument	Requested days	Allocated days	From	To
D16	7	6	11/06/2021	17/06/2021

Abstract:

The lipids found in archaeal membranes differ from those found in bacteria in several ways. One of these differences is the presence of ether rather than ester linkages (typically found in bacterial membranes) between the hydrocarbon tails and the glycerol backbone. Ether bonds are thought to provide an advantage in high temperature environments where many archaea live. In this work we wish to probe the differences in the membrane structure of ester linked lipids, analogous to ether linked archaeal lipids previously characterized, as a function of both hydration and temperature. This will tell us the relative contribution of this type of linkage on membrane parameters and may give insights into the importance of the ether linkage in archaeal lipids.

Experiment# 9-13-1034: Role of ether versus ester bonds in the hydration and temperature stability of branched chain lipids

Introduction: Archaea have lipids which are distinct from the other two domains of life. First, the lipid tails are isoprene-based resulting in methyl branching unlike the straight acyl chains typically found in other organisms. These tails are linked to the glycerol backbone via ether bonds rather than ester bonds and finally archaeal lipids have the opposite stereochemistry (G1P) compared to bacterial/eukaryotic lipids (G3P) [1-2]. In this work we probed the **ester-analogs (diphytanoyl)** of the **ether-linked (diphytanyl)** lipids which we have previously characterized. By studying these analogs differing only by the nature of the ester/ether linkage it will be possible to test experimentally the relative contribution of this linkage on membrane parameters. Lipids with three different headgroups were compared, PC (phosphocholine), PS (phosphoserine) and PG (phosphoglycerol). *Note: all lipids discussed in this report have (R, G3P or "bacterial") backbone stereochemistry with the exception of DoPhPG which is a racemic mixture of (R/S) stereochemistry.*

Sample Preparation: Oriented membranes stacks were prepared on ultraclean silicon wafers by depositing 3 mg of lipid in a solution of 2:1 chloroform:methanol. The solvent was evaporated under vacuum overnight and rehydrated at the corresponding ratio of D_2O:H_2O. To precisely control the humidity of the sample we used the BerILL humidity chambers.

Results: Initially diffraction was measured using a 100% D_2O contrast for ester-linked lipids (DPhPC, DPhPS, DPhPG) and ether-linked lipids (DoPhPC, DoPhPS, DoPhPG) containing the same polar headgroups and same hydrocarbon chains. All of the ester-linked lipids formed well ordered lipid multistacks which gave up to 4 orders of diffraction. Interestingly, only the ether-linked lipid with the PC headgroup formed well-ordered stacks. DoPhPS and DoPhPG had much more poorly ordered stacks resulting in only a single order of diffraction (Figure 1). This was true at all humidities tested.

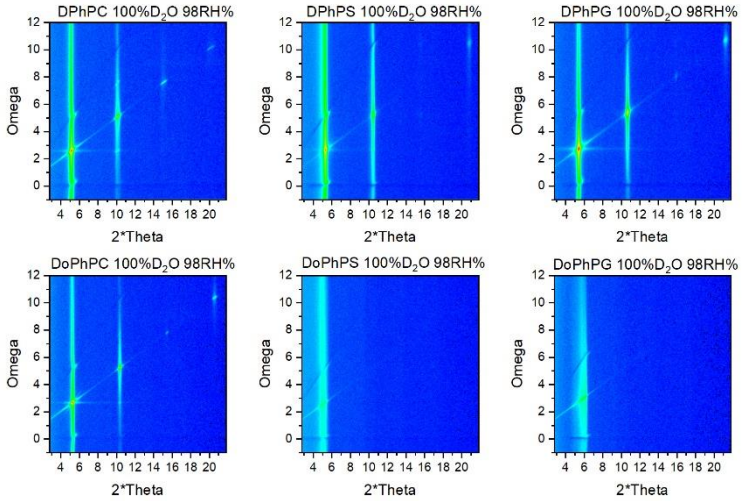

Figure 1: 2D diffractograms taken at 98%RH and 100% D_2O contrast.

From the location of the Bragg peaks we can determine the lamellar repeat spacing (d-spacing) which is composed of the thickness of a single bilayer and the associated water layer. The d-spacing was calculated for strongly diffracting lipid multistacks as a function of humidity from 90 – 100 %RH (Figure 2). An increase in d-spacing can be seen for all lipids as a function of humidity. DoPhPC was the only ether which gave multiple diffraction peaks. When comparing DoPhPC to its ester analog, DPhPC, there is a clear difference in the d-spacing between these two lipids, with the ester being ~1-2 Å larger. All three esters gave multiple orders of diffraction. The different polar headgroups did affect the d-spacing of the otherwise identical lipids. DPhPC had the largest d-spacing, followed by DPhPS and DPhPG had the smallest d-spacing.

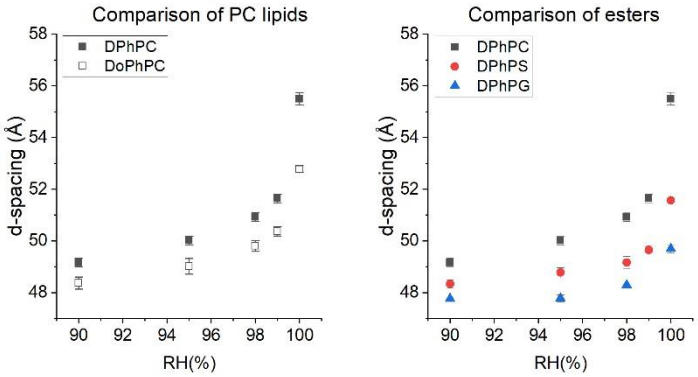

Figure 2: d-spacing of lipid multistacks measured at 100% D_2O contrast. (Left) Comparison of DPhPC (ester) and DoPhPC (ether) as a function of humidity. (Right) Comparison of diphytanoyl lipids containing different polar headgroups (PC, PS, PG) as a function of humidity.

The PC lipids were then measured at different %D_2O to improve the contrast and in order to help define the phase of the structure factor phases necessary to construct neutron scattering length density plots (NSLD). NSLD plots were constructed for DPhPC and DoPhPC using the diffraction data at 8% D_2O which gives the best contrast between the water and lipid. The NSLD plots were then used to determine further structural parameters such as the bilayer thickness (d_B), water layer thickness (d_w), and lipid area (A) (Table 1). The bilayer thickness for the ester was found to be smaller than the ether, and the lipid area was larger. The most striking difference between the two is probably the increased water layer thickness for the ester, which becomes even more pronounced at high humidity.

Table 1: membrane parameters for PC-lipids as a function of humidity (8% D_2O contrast)

	DPhPC (ester)					DoPhPC (ether)				
	90% RH	95% RH	98%RH	99%RH	100%RH	90% RH	95% RH	98%RH	99%RH	100%RH
d	49.9 ± 0.1	50.7 ± 0.2	51.4 ± 0.2	52.2 ± 0.3	57.4 ± 0.1	48.7 ± 0.2	49.5 ± 0.2	50.3 ± 0.2	50.9 ± 0.2	52.0 ± 0.2
d_B	34.9	34.7	34.2	34.1	33.2	36.1	36.3	36.5	36.2	36.4
d_w	15.0	15.9	17.2	18.1	24.1	12.7	13.2	13.8	14.7	15.6
A	80.4	80.9	82.2	82.4	84.6	75.3	74.9	74.4	75.0	74.6

Discussion: The first interesting finding of this study was the fact that the ability of lipids to form well-ordered stacks is influenced by both the polar headgroup and the type of linkage found between the lipid tails and the glycerol backbone. While the PC ether- and ester-linked lipids both formed well-ordered stacks as evidenced by the multiple orders of diffraction, the ether linked lipids with either a PS or PG headgroup did not form well-ordered stacks. The poor diffraction from these lipids made it impossible to do a detailed comparison of the membrane parameters PS/PG ether vs esters. The d-spacing can be estimated from a single Bragg peaks for lipids in which only a single peak as present, however these results are not discussed here.

The increased lipid area and water layer thickness for the ester can be attributed to the presence of the carbonyl oxygen as the lipids are otherwise identical. These findings are in agreement with molecular dynamics studies [3-5]. It has been proposed that, by being less polar and smaller, the **ether** bonds lead to a higher compaction of the lipid molecules in the membrane compared to the traditional **ester** linkages. Indeed, the larger lipid area for the ester-linked lipid indicates that this lipid forms a less compact bilayer. This compaction is thought to lead to higher stability for the ether-linked lipids in extreme conditions. It is also interesting to note that the lipid area for the ester also appears to increase with humidity as the lipid becomes more hydrated, but that the ether lipid remains relatively constant with increasing humidity.

References: [1] Koga & Morii (2007) *Microbiol. Mol. Biol. Rev.*, [2] Siliakus et al. (2017) *Extremophiles*, [3] Shinoda et al. (2004) *J. Chem. Phys.*, [4] Kruczek et al. (2017) *BBA*, [5] Rasouli et al. (2021) *J. Mech. Behav. Biomed. Mater*.