Experimental report

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Proposal: 8-02-898 **Council:** 10/2019

Title: Enantiomeric study on archaeal lipids

Research area: Biology

This proposal is a new proposal

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Samples: left-handed 1,2-di-O-phytanyl-sn-glycero-3-phosphoserine (DoPhPS)
racemic 1,2-di-O-phytanyl-sn-glycero-3-phosphoserine (DoPhPS)
right-handed 1,2-di-O-phytanyl-sn-glycero-3-phosphoserine (DoPhPS)

Instrument	Requested days	Allocated days	From	To
IN15	6	5	09/02/2021	14/02/2021

Abstract:

Nature's selection of only right-handed sugars and left-handed amino acids upon which to build life is still not understood, nor are the consequences of these facts. In the present study, we would like to investigate the bending rigidity of archaeal lipids of the two types of chirality and their mixture to shed light on the question whether these structural characteristics might have effects on the membrane architecture of Archaea.

8-02-898: Enantiomeric study on archaeal lipids

Introduction: Membrane phospholipids are chiral molecules, principally with respect to the glycerol backbone. In bacteria and eukaryotes, the lipids are exclusively of the glycerol-3-phosphate (G3P) stereochemistry, whereas in archaea the glycerol backbones are entirely of the glycerol-1-phosphate (G1P) stereochemistry. Understanding the role of lipid stereochemistry on membrane behavior could give insights into this "lipid divide". Heterochiral membranes are known to be stable, and it has been hypothesized that early life could have had such "mixed" membranes [1-3]. If no other chiral centers are present, lipids that differ only by the stereochemistry of the glycerol backbone are enantiomers (mirror images of each other). If other chiral centers are present, and the lipids differ only by the backbone stereochemistry, these lipids are diastereoisomers (non-enantiomeric isomers). While enantiomers are thought to be to be equivalent (with the exception of chiral properties), disastereoisomers can have different properties. As part of the ANR Archaeomembranes project we synthesized archaeol based lipids with different polar headgroups: 1,2-diphytanyl-sn-glycero-3-phospho-L-serine (DoPhPS-(R)); 2,3-diphytanyl-sn-glycero-1-phospho-L-serine (DoPhPS-(S)); and an equimolar mixture of the two diastereoisomers (DoPhPSrac); 1,2-diphytanyl-glycero-phospho-1'-rac-glycerol (DoPhPG) and 1,2-diphytanyl-glycero-phospho-1'-myo-inositol (DoPhPI). All the phospholipids in this study are archaeol based with identical phytanyl chains linked to the glycerol backbone via ether bonds. R, S and rac (racemic) are used in the naming to refer to the stereochemistry of the glycerol backbone. Difficulty forming vesicles from the DoPhPS diastereoisomers, DoPhPG, or DoPhPI alone prompted the use of mixtures with 1,2-diphytanyl-sn-glycero-3-phosphocholine (DoPhPC). MLVs were formed in 50 mM Tris pH 7.4 (D_2O) for a final lipid concentration of 10 mg/ml (~12 mM) and passed 11x through a 0.1 µm (100 nm) filter to form ULVs. The effect of the polar headgroup and the backbone stereochemistry on archaeal membrane dynamics was probed via neutron spin echo (NSE) and dynamic light scattering (DLS) at a range of temperatures between 25 and 85°C.

Results: DLS data was analyzed using the method of cumulants [4] and the vesicle hydrodynamic radius (R_h) determined through the Stokes-Einstein relation (Figure 1 left). At 25°C, the ULVs of DoPhPC where found to have a R_h of ~74 nm. The 1:1 mixtures of DoPhPC with each of the DoPhPS diastereoisomers produced ULVs of a similar size, whereas the presence either DoPhPG or DoPhPI led to larger ULVs (Table 1). All samples showed a slight increase in the vesicle R_h as the temperature increases which has been seen previously with other vesicles systems. Interestingly, the R_h of the PS-(S):PC (1:1) membrane began to increase much more rapidly ~55°C (and PI:PC (1:2) to a lesser extent).

Table 1: Hydrodynamic radius (R_h). Values are average ± stdev of four measurements on the same sample.

	25°C	40°C	55°C	70°C	85°C
DoPhPC	74.1 ± 0.5	77.3 ± 1.4	80.7 ± 0.4	85.0 ± 0.8	91.1 ± 1.4
PS-(R):PC (1:1)	70.7 ± 0.1	74.7 ± 0.4	77.7 ± 0.1	79.0 ± 0.2	81.1 ± 0.5
PS-(S):PC (1:1)	75.6 ± 0.2	80.4 ± 0.6	90.2 ± 2.0	126.9 ± 9.6	--
PSrac:PC (1:1)	77.1 ± 1.5	80.5 ± 0.3	84.1 ± 0.1	85.8 ± 0.3	--
PG:PC (1:1)	91.4 ± 0.7	94.6 ± 0.1	94.4 ± 0.2	97.2 ± 2.1	--
PI:PC (1:2)	82.0 ± 0.3	87.3 ± 0.3	99.0 ± 2.5	115.7 ± 4.0	--
PI:PC (1:1)	85.7 ± 0.1	90.0 ± 0.2	95.6 ± 0.4	102.9 ± 1.6	Agg.
PI:PC (2:1)	106.3 ± 0.2	110.4 ± 0.3	109.2 ± 0.2	108.6 ± 1.2	--
DMPC	71.5 ± 0.2	76.1 ± 0.1	79.7 ± 0.2	81.5 ± 0.4	--

Neutron spin echo data was analyzed following the Zilman-Granek model [5]. An example of the fits can be seen in Figure 1 (right). The intermediate scattering function (S(Q,t)) was fit to a stretched exponential where Γ_{ZG} is the q-dependent decay rate :

$$S(Q,t) = \exp(-(\Gamma_{ZG}t)^{\frac{2}{3}})$$

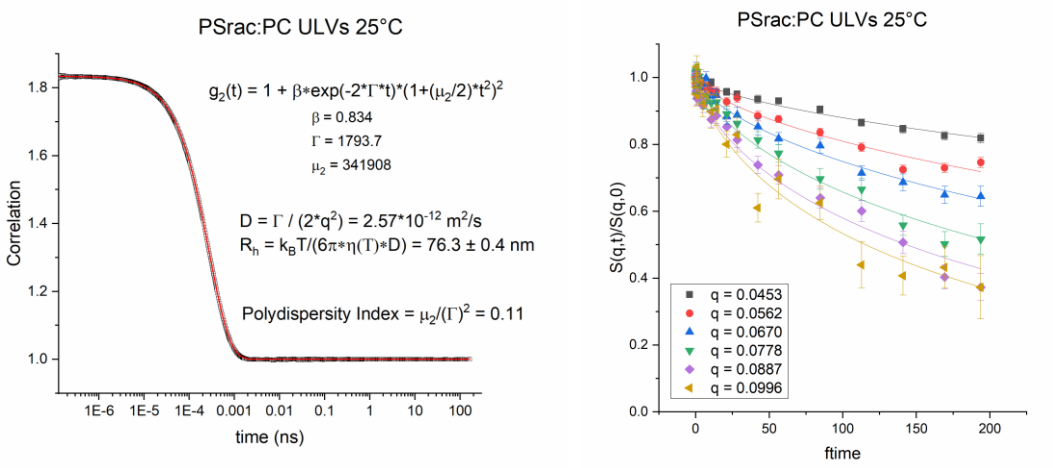

Figure 1: Example of fitting for DLS data (left) and NSE data (right). ULVs were formed from a mixture of DoPhPC:DoPhPSrac (1:1) in 50 mM Tris pH 7.4 (D_2O). Lipid concentration was 10 mg/ml. Measurements were performed at 25°C.

Relaxation proportional to Q^3 is typical of lipid bending modes. The decay rate is related to the bending modulus (κ) though the following relationship:

$$\frac{\Gamma_{ZG}}{Q^3} = 0.0069\,\gamma\,\frac{k_BT}{\eta(T)}\sqrt{\frac{k_BT}{\kappa}}$$, where γ=1 for κ>>k_BT [6,7].

The calculated values of the membrane bending rigidity can be found in Table 2. As expected, the membrane rigidities tend to decrease as a function of temperature. Mixtures of each of the DoPhPS diastereoisomers with DoPhPC showed similar values for the bending rigidity at low temperatures (~21 k_BT at 25°C and ~16 k_BT at 40°C). At 55°C or above, the PS-(S):PC sample showed deviation from the expected behavior making it difficult to determine an accurate bending rigidity. This is the same temperature at which we start to see the dramatic change in vesicle size. The PG:PC membrane was the most rigid of the 1:1 lipid mixtures tested here. Previous work with mixtures of POPG and POPC showed a stiffening of the membrane from ~28 k_BT for POPC alone to ~50 k_BT for 50% POPG [8]. Mixtures containing different quantities of DoPhPI also showed higher rigidities with the 2:1 ratio of PI:PC being significantly more rigid than either the 1:1 or 1:2 mixtures. When diffusion (determined via DLS) was accounted for in the analysis of S(Q,t) similar trends in rigidity were observed though the absolute magnitude of the bending rigidities were found to be higher than when diffusion was ignored.

Table 2: Membrane bending rigidity (k_BT). Rigidity and errors were determined based on the average and stdev of Γ_{ZG}/Q^3 calculated for each value of Q.

	25°C	40°C	55°C	70°C	85°C
PS-(R):PC (1:1)	20.9 ± 0.8	16.0 ± 0.9	11.9 ± 0.6	9.5 ± 0.3	7.9 ± 0.3
PS-(S):PC (1:1)	22.6 ± 2.8	16.7 ± 1.2	15.5 ± 0.6	**	n.d.
PSrac:PC (1:1)	21.9 ± 1.7	16.9 ± 1.1	13.4 ± 0.7	10.5 ± 0.4	n.d.
PG:PC (1:1)	43.5 ± 4.4	31.6 ± 5.3	23.4 ± 2.6*	20.6 ± 0.7*	n.d.
PI:PC (1:2)	27.4 ± 1.7	21.4 ± 1.3	17.5 ± 0.2	15.7 ± 0.4	n.d.
PI:PC (1:1)	28.3 ± 2.1	20.3 ± 1.0	17.1 ± 1.1	14.6 ± 0.5	Agg.
PI:PC (2:1)	50.3 ± 9.2	35.3 ± 4.7	**	**	n.d.

*high-q data excluded due to poor statistics; ** deviated from expected behavior for bending modes

DoPhPC and DMPC ULVs did not show the expected Q^3 dependence of Γ_{ZG} and further analysis will be needed for these samples to characterize their dynamics. Other memebrane systems have shown similar behavior and models have been proposed to explain this type of behavior [9,10].

References: [1] Koga (2011) *J. Mol. Evol.*; [2] Shimada & Yamagishi (2011) *Biochemistry*; [3] Caforio et al. (2018) *P.N.A.S.*; [4] Frisken (2001) *Appl. Opt.*; [5] Zilman & Granek (1996) *Phys. Rev. Lett.*; [6] Nagao et al. (2017) *J. Phys. Chem. Lett.*; [7] Hoffmann et al. (2014) *Nanoscale*; [8] Faizi et al. (2019) *Soft Matter* ; [9] Arriaga et al. (2010) *Eur. Phys. J. E. ;* [10] Hoffmann (2021) *Frontiers in Physics*