Experimental report

24/08/2021

Proposal: 8-02-952 **Council:** 10/2020

Title: Salinity adaptation in halophilic archaeal membranes

Research area: Biology

This proposal is a new proposal

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Samples: natural extracted archaeal lipids

Instrument	Requested days	Allocated days	From	To
D16	8	6	08/07/2021	14/07/2021

Abstract:

Extreme halophiles can grow in conditions where the activity of water is close to 0. Despite an increased intracelullar osmolarity, a strong gradient exists between the environment and the cell, and the physico-chemical tolerance of the plasmic membrane to this osmotic stress remains unknown. Part of the increased membrane stability has been associated to the presence of two kinds of unusual archaeal lipids which have extended hydrophobic cores. These lipids could form a zipped bilayer membrane in which lipids from both faces would interact inside the midplane, with increased membrane parameter values. In this experiment, we will study reconstructed archaeal membranes to determine the impact of the proportion of extended archaeol on membrane structure and membrane resistance to high temperatures. Using these lipids, we will be able to evaluate the relative contribution of the extended lipids in membrane physical parameters in the archaeal bilayer by comparison with the 'classic' archaeal bilayer, which has already been extensively characterized.

Experiment #8-02-952: Salinity adaptation in halophilic archaeal membranes

Introduction: Halophilic organisms can grow in solutions of up to ≥20% (w/v) salt. It is thus very important to have a membrane that can act as an effective barrier under these conditions. Halophilic archaea have been associated with the presence of two kinds of unusual lipids which have extended isoprene-based hydrocarbon chains ($C_{25}C_{20}$ and $C_{25}C_{25}$) in addition to the typical $C_{20}C_{20}$. Lipids were extracted from three different archaea: *Haloferax volcanii* (37°C, pH 7.0, 12.5% NaCl) which consists of 100% $C_{20}C_{20}$ carbon chains, *Natronomonas pharaonis* (37°C, pH 9.0, 20.0% NaCl) which consist of about 90% $C_{25}C_{20}$chains, 10% $C_{20}C_{20}$ chains and trace amounts of $C_{25}C_{25}$ chains, and *Aeropyrum pernix* (90°C, pH 7.0, 2.0% NaCl) which consist of about 98% $C_{25}C_{25}$chains, 2% $C_{25}C_{20}$ chains and trace amounts of $C_{20}C_{20}$ chains. The extracted lipids were separated using thin layer chromatography and lipids with several different polar headgroups including phosphohexose (PH), phosphoglycerol (PG) and phosphoglycerolmethylphosphate (PGPMe) were detected via mass spectrometry. *Aeropyrum pernix* (Ape) fraction #1 is composed of $C_{25}C_{25}$-PH. *Natronomonas pharaonis* (Nph) fraction 1&2 are composed of $C_{25}C_{20}$-PG : $C_{20}C_{20}$-PG (9:1) and fraction 3 is composed of $C_{25}C_{20}$-PGPMe : $C_{20}C_{20}$-PGPMe (9:1). *Haloferax volcanii* (Hvol) fractions 1&2 are composed of $C_{20}C_{20}$-PG and fractions 3,4 &5 are composed of $C_{20}C_{20}$-PGPMe. We are interested in characterizing structural differences based on both the length of the isoprene-based chains and the polar headgroup composition.

Results: It was possible to form oriented multistacks from lipids extracted from archaea which were sufficiently ordered for neutron diffraction experiments. The extracted lipids containing a PG headgroup gave two orders of diffraction (at 25°C) for lipids extracted from both *Haloferax volcanii* (Hvol) and *Natronomonas pharaonis* (Nph) and a 50:50 mixture of the two (Figure 1). Hvol f2 lipid consists of solely $C_{20}C_{20}$ chains and Nph f1 contains a (9:1) ratio of $C_{25}C_{20}$ and $C_{20}C_{20}$ chains. While the Nph f1 appears to form a single lamellar phase, the Hvol f2 and PGmix each exhibit at least two lamellar phases. The Hvol f2 membranes give much broader peaks in the omega direction.

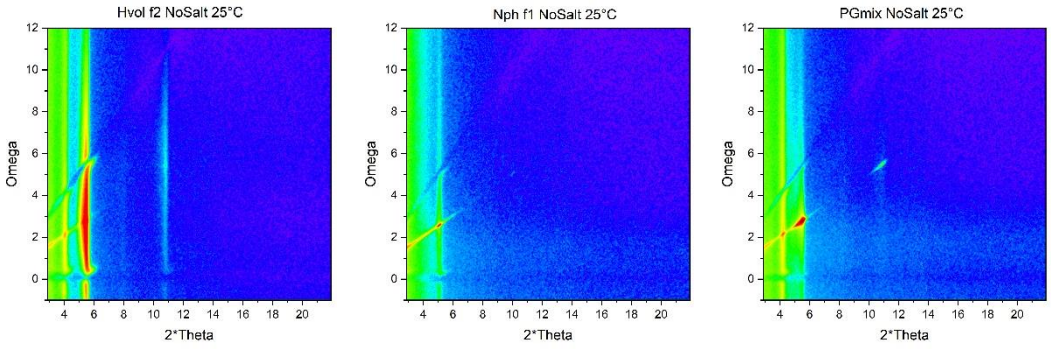

Figure 1: 2D diffractograms of lipids with PG polar headgroups extracted from Hvol (left), Nph (center) or a mixture of the two (right). Diffraction performed in flat aluminum cells.

The d-spacing of these membrane phases were determined for each of these three membranes at temperatures between 25 and 85°C (Table 1).

Table 1: d-spacing of lipids with PG polar headgroups extracted from halophilic archaea

	25°C	40°C	55°C	70°C	80°C
Hvol f2 Phase 1	63.4	64.4	65.9	--	--
Hvol f2 Phase 2	47.3	48.8	50.4	--	--
Nph f1 Phase 1	50.4	--	--	--	--
PGmix Phase 1	75.2	74.9	72.9	72.4	75.8
PGmix Phase 2	70.0	61.1	60.7	61.2	62.9
PGmix Phase 3	47.0	47.5	49.1	53.6	--

To improve diffraction for the lipids that did not diffract well in the flat cells, we used the BerILL chambers to look diffraction at lower humidities. We also changed the detector position to determine if there were peaks at lower q values. We were able to detect some diffraction for both Nph and Hvol lipids with the PGPMe headgroup but the diffraction was not very strong (Figure 2). The d-spacing was determined based on the location of the first order diffraction peaks.

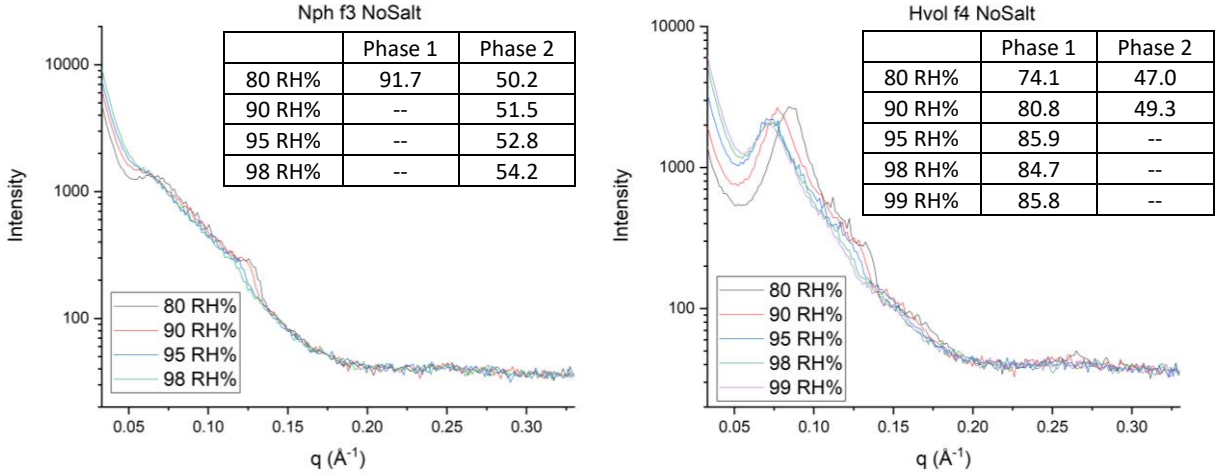

Figure 2: 1D diffractograms of lipids containing PGPMe headgroups from N. pharaonis (left) and H. volcanii (right). Nph has mainly $C_{25}C_{20}$ chains and Hvol has $C_{20}C_{20}$ chains. Diffraction performed in BerILL chambers.

The lipids extracted from *Aeropyrum pernix* had different headgroups from those seen in the other two organisms. This may be partially because *A. pernix* grows in oceanic salt concentrations rather than extreme salt conditions. It is perhaps unsurprising that the halophile specific headgroup PGPMe and the related PG were not found in the lipids extracted from this organism. Only a single headgroup (phosphohexose, PH) was detected. Ape f1 has mainly $C_{25}C_{25}$ lipid chains but the effect of these chains can't be compared with the $C_{25}C_{20}$ and $C_{20}C_{20}$ lipids extracted from Hvol and Nph due to the difference in headgroup composition.

The Ape f1 diffracted poorly in the flat cell, giving only a single weak diffraction peak (~54.5 Å) so this membrane was remeasured in the BerILL humidity chamber. The diffraction signal was much improved at in the BerILL chamber at humidities between 80 and 98%. Two phases are seen (Figure 3). Phase 1 which corresponds to a d-spacing of >80Å and Phase 2 which has a d-spacing ~50Å. Both phases increase in d-spacing with increasing humidity as expect.

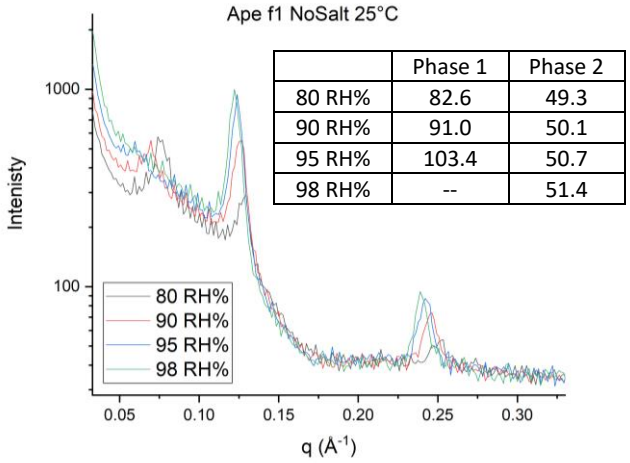

Figure 3: 1D diffractograms of lipids extracted from Ape at different humidities. These lipids have $C_{25}C_{25}$ chains. Diffraction was performed in BerILL chambers.