Experimental Report

Proposal: **8-04-691** **Council:** 10/2012

Title: Molecular adaptation of plasmic membrane in Archael Piezophile

This proposal is continuation of: 8-04-655

Researh Area: Biology

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Samples: Membranes from E coli and T barophilus

Instrument	Req. Days	All. Days	From	To
IN13	8	6	29/04/2013	02/05/2013

Abstract:

In the past, neutron experiments proved to be successful in quantifying the extent to which macromolecular dynamics in bacterial cells is affected by adaptation to extreme temperatures and salinity (Tehei et al., 2004; Tehei et al., 2007). The results supported the hypothesis that the evolutionary selection of appropriate resilience in order to maintain macromolecular structure and flexibility within the narrow limits required by biological activity contributes to environmental adaptation. We investigated recently the effect of high hydrostatic pressure on lipid model membranes. Due to the pressure induced order, the main phase transition between the gel and the liquid state in such membranes is shifted to higher temperatures under high pressure (Peters et al., 2011). To maintain the fluidity in the membrane, more unsaturated lipids are found in organisms from deep sea environments.

The objective of our project is to compare the dynamics of plasma membranes of procaryotes adapted to different environmental conditions (temperature and pressure) and to study by this way the mechanisms of environmental adaptation of biological systems.

Report on April/May 2013 IN13 experiment 8-04-691 and CRG 2031

(J. Peters, N. Martinez, A. Cario, P. Oger)

Molecular adaptation of plasmic membrane in Archael Piezophile

Recent neutron experiments supported the hypothesis that the evolutionary selection of appropriate resilience in order to maintain macromolecular structure and flexibility within the narrow limits required by biological activity contributes to environmental adaptation. To verify that, we investigated already the effect of high hydrostatic pressure on multilamellar vesicles composed by pure 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) (1). Due to the pressure-induced order, the main phase transition between the gel and the liquid state in such multi-lamellar structures is shifted to higher temperatures under high pressure.

The objective of the present project was to compare the dynamics of plasma membranes of procaryotes adapted to different environmental conditions and to study by this way the mechanisms of environmental adaptation of biological systems. We proposed to measure membranes prepared from cultures grown at different pressure conditions to address validity of the relationship between the modification of plasma membrane composition and membrane fluidity in connection to high hydrostatic pressure (HHP) adaptation, a mechanism commonly referred to as "homeoviscous adaptation" (2). However, due to the large quantities of sample required for analyses on the instrument IN13, as well as the lack of a proper set of data on a model membrane reference for mesophilic organisms and in order to establish the most appropriate protocols for sample preparation, we started in 2012 (3) with lipids extracted from a mesophilic control organism, i.e. from *Escherichia coli* (*E. coli*). The sample contained a distribution of intact polar lipids corresponding to the natural lipid mixture in *E. coli* membranes, i.e. C16 to C18 fatty acids with no or one insaturation on the fatty acid chains, and phosphatidylacetylcholine as the major polar head group. In 2013 we had a sample from *Thermococcus barophilus (T. barophilus)* in addition.

The first experiment undertaken in 2012 (3) on lipids extracted from *E. coli* revealed that the slopes of the summed intensities as function of temperature were the same when measured in a standard flat sample holder or in the cylindrical high pressure cell (see figure 1) (4). Unfortunately, we could not go to temperatures much higher than 310 K with the pressure cell as the Aluminum does not support it well.

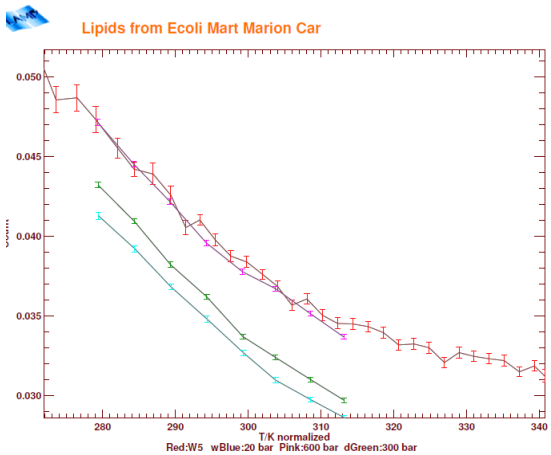

Figure 1: Summed intensities in arbitrary units of lipids extracted from *E. coli* at 1 bar (in red, measured in a standard sample holder), at 20 bar (in pink), 300 bar (in green) and 600 bar (in blue).

We remarked, however, a change in slope of the summed intensity around 315 K (when measured in the flat sample holder at 1 bar). As phase transitions are shifted in temperature by pressure application (1), we wanted now to check whether it would be possible to see such an effect on the lipids when pressure is applied and decided to try to go at least to 340 K with the high pressure cell. Such a measurement required a heating insert inside of the closed cycle dry cryostat of IN13, which was found to be not compatible with the height of the high pressure can. We then tried to build up an adapter and were able to go to higher temperature, but the high pressure cell was indeed breaking at 336 K and part of the sample was lost. So we got data only at low temperature for *T. barophilus* and data at high temperature for *E. coli* (see figure 2). Unfortunately, the statistics and the limited temperature range did not allow a reasonable conclusion about possible changes of the slope of the summed intensities.

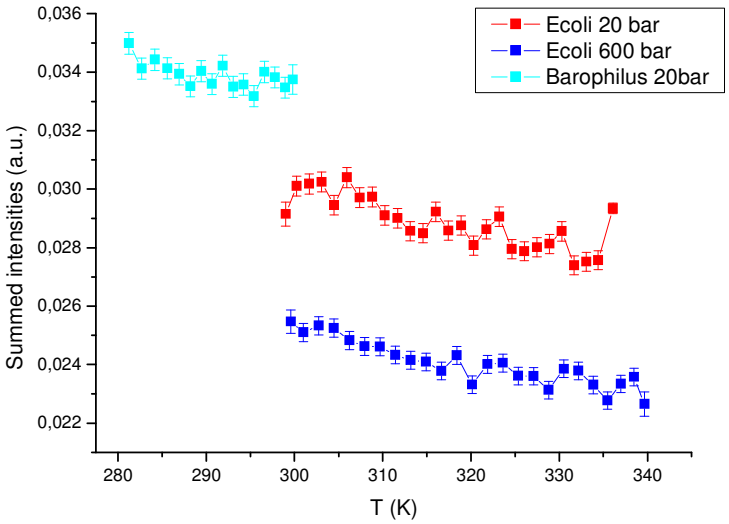

Figure 2: Summed intensities in arbitrary units of lipids extracted from *E. coli* at 20 bar (in red), and 600 bar (in blue) and from *T. barophilus* at 20 bar (in cyan).

Therefore we would like to measure now lipids extracted from *T. barophilus* first in a flat sample holder up to high temperature (< 370 K) to determine if there is a pressure range where changes of slopes might happen. Furthermore, we submitted an NMR proposal to better specify the exact lipidic composition in *T. barophilus* to the CBMN-IECB Bordeaux.

References

(1) Trapp et al., Phys. Chem. Chem. Phys. 15 (2013), 20951.
(2) Sinensky, PNAS 71 (1974), 522-525
(3) ILL report 8-04-655
(4) Peters et al., High Pressure Research 32 (2012), 97 - 102.