Experimental Report

Proposal: **9-13-496** **Council:** 10/2012

Title: Confinement effects on lipids withcharged headgroups.

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DPPG)
1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

Instrument	Req. Days	All. Days	From	To
D17	4	3	02/08/2013	05/08/2013

Abstract:

Supported lipid bi-layers are an excellent model system for biological membranes. The mechanical properties of these layers are highly relevant to their functionality, for example determining how easily proteins and small molecules can insert themselves into the layer. We have recently developed a unique surface force style apparatus that allows direct measurements of the structure of thin layers under confinement using neutron radiation. Our recent ILL experiment determined that the hydration and phase behaviour of certain lipids changes under confinement, but that these changes are approximately constant for the phosphocholine zwitterionic head group with different tail saturation and lengths. This indicates that the lipid head group determines the response of the bilayers to confinement. We propose to study two model lipids with identical hydrocarbon tails, but differently charged head groups, both individually and as mixtures. This will allow us to better understand the role of the head group and look at mixtures of lipids which are more biologically relevant.

Title: Further effects of confinement on lipid bilayers.

Experiment: 9-13-496 Dates: 02/08/2013 - 05/08/2013 Instrument: D17
Team: L.L.E. Mears, R. Barker, S.B. Abbott, S.W. Prescott, W.M. de Vos, R.M. Richardson.
Local contact: R. Barker

Abstract

Previous ILL experiments, on the confinement of lipid bilayer stacks, showed an interesting difference in the behaviour of the lipid DPPC under confinement in the gel and fluid phases. In the gel phase the interlayer spacing decreased whereas in the fluid phase it increased indicative of the DPPC undergoing a confinement induced phase transition. In contrast, the DMPC did not appear to show a thickening upon confinement from the fluid phase. This experiment was used to build a comprehensive overview of the effect tail length and starting hydration state has on the behaviour of phosphatidylcholine lipids under confinement. Thus, conclusions about the change in phase behaviour can be made with more certainty, when combined with the previous experiments.

Introduction

Lipid bilayers are frequently used as a model system without the full complexity of cell membranes. Their phase behaviour is influenced by environmental conditions, such as temperature, pressure and hydration. In a previous experiment using the D17 reflectometer [1] our novel surface force type apparatus [2] was used to probe the effect confinement has on spin coated lipid bilayer stacks. In those experiments hydrogenous phosphatidylcholine lipids, of different tail lengths, were hydrated with D_2O vapour prior to confinement. The data suggested that under confinement some of this D_2O is removed from the stack, a consistent feature for all the lipids in both the fluid (L_α) and gel (L_β) phases. For lipids in the gel phase the shift in Bragg peak position indicated an associated thinning of the layers. However for DPPC, the lipid with the longest tail length which we looked at in the fluid phase during that experiment, there was a surprising increase in the repeat distance. In a further experiment to probe the temperature dependence in more detail DMPC was used [3], the samples were also hydrated directly with liquid D_2O applied to the surface. The DMPC did not show a thickening in the fluid phase in the same way as DPPC but instead the layers thinned, similar to DLPC.

It was originally intended that this experiment would expand our study to the headgroup's effect on the lipid phase behaviour under confinement, by using charged headgroups with the same tail length. However, having only seen the thickening behaviour in DPPC, starting from the vapour hydrated state, further data was needed to fully understand this behaviour and the data sets already taken before we can pursue other factors. Hence the following experiment was undertaken.

Experimental results

Samples were prepared by spin coating from chloroform onto 3" Si blocks, to form ~8 bilayers (40-50nm) of the hydrogenous lipids 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine

(DMPC), 1,2-palmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). D_2O was used in order to enhance the contrast while either fully hydrating the samples or partially hydrating them with vapour. Samples were confined at different temperatures in either the gel or fluid phase. For two samples (DPPC fully hydrated with D_2O and DSPC hydrated with D_2O vapour) the Bragg peak position was also monitored while it was cooled, under confinement, from the fluid phase to a temperature that would be within its gel phase under ambient conditions.

The reflectivity from confined DSPC, in the fluid phase at 70°C and hydrated with D_2O vapour, is shown in figure 1. Notably the interlayer spacing increases just as it did for the DPPC in our previous experiment. Further analysis of the distribution of water in the layer using model fitting is ongoing.

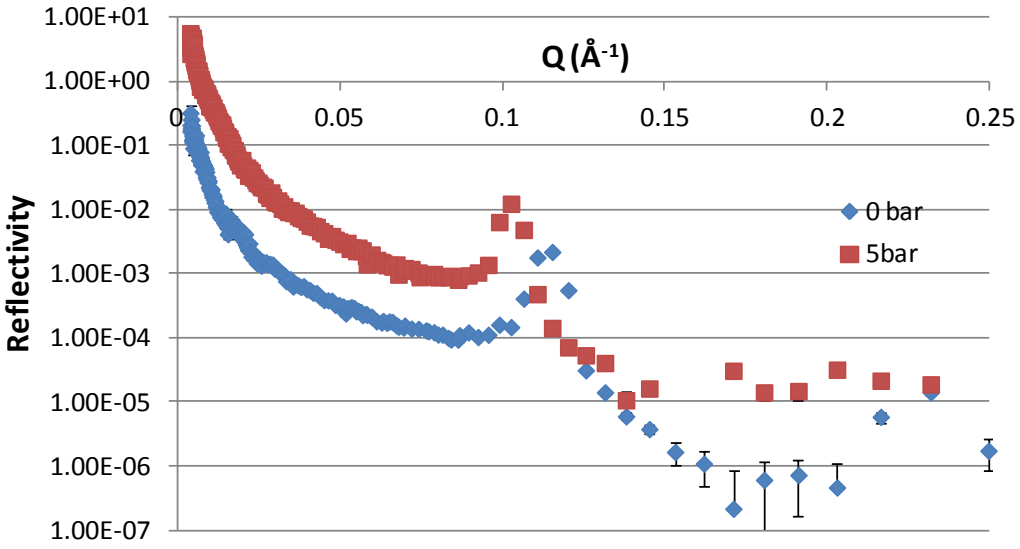

Figure 1 Reflectivity profiles for DSPC at 70°C hydrated with D_2O vapour without confinement, 0 bar and under 5 bar of confining pressure. The 5 bar data has been offset for clarity and shows the shift of the Bragg peak to lower Q (higher spacing) on confinement.

Conclusions

The experiment has given more information about the effect of confinement on the structure of phosphatidylcholine lipid bilayer stacks in both the gel and fluid phases of lipids with different tail lengths. It has also touched on differences between different hydration starting points and how the interlayer spacing responds to cooling once confined. Further analysis will be carried out, on both the data from this experiment and from the previous experiments, to give a comprehensive overview of the phase behaviour of lipids under confinement.

References

1. W.M. de Vos, L. L. E. Mears, S. W. Prescott, R. M. Richardson, T. Cosgrove, and R. Barker, *Exp. report ILL, D17*, 9-13-375 (2011).
2. W.M. de Vos, L.L.E. Mears, R.M. Richardson, T. Cosgrove, R.M. Dalgliesh, S.W. Prescott, *Rev. Sci. Instrum.*, 83, 113903 (2012).
3. L. L. E. Mears, W.M. de Vos, S. B. Abbott, S. W. Prescott, T. Cosgrove, R. M. Richardson, and R. Barker, *Exp. report ILL, D17*, 9-13-454 (2012).
4. L. L. E. Mears, S. B. Abbott, W.M. de Vos, S. W. Prescott, T. Cosgrove, R. M. Richardson, and R. Barker, *Exp. report ILL, D17*, 9-13-518 (2013).