Experimental report

22/10/2015

Proposal: **8-02-708** **Council:** 10/2014

Title: How do neutrons see phosphatidyl serine? Ca-bound and Ca-free forms of PS studied by neutron reflectometry at the air-water interface.

Research area: Other...

This proposal is a resubmission of 8-02-697

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Experimental team: Ilya REVIAKINE

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Samples: C34H11NO10PNaD54

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	3	2	13/10/2015	15/10/2015

Abstract:

Phosphatidyl serine (PS) is a phospholipid that is crucially important in blood clotting and cell membrane repair. It has a strong affinity for Ca2+ and serves as a binding site for a variety of proteins (e.g., clotting factors, annexins), both in the Ca-free and Ca-dependent manner. While it has been extensively studied by a variety of spectroscopic methods and extensively used in various membrane model systems to investigate cell membrane behavior and lipid-protein interactions, precious little work has been done by neutron reflectometry (NR) to characterize this lipid. Crucial physico-chemical parameters of Ca-bound PS headgroup are not known, and questions about its interactions with other lipids remain. NR experiments at the air-water interface proposed here are aimed at characterizing PS headgroup in the Ca-bound and Ca-free form to fill that gap in our knowledge.

Report 8-02-708
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Ca-bound and Ca-free forms of PS studied by neutron reflectometry at the air-water interface.

Phosphatidyl serine participates in such physiological processes as clot formation,[1] cell membrane repair,[2] signaling in apoptosis,[3] biomineralization,[4] and cytoskeleton-membrane interactions.[5] Many of its physiological roles depend on its ability to bind calcium. For example, clotting factors bind PS in a Ca-independent manner,[1] while the binding of annexin A5,[6] a protein involved in cell membrane repair,[2] is Ca-dependent. Despite its importance, structural information on PS lipids, either in the Ca-free or Ca-bound form is scarce. Langmuir trough studies conducted in late 1970s and 1980s established that the ion binds to the phosphate group in the lipid, causes a conformational change, and has a condensing effect on the lipid, reducing its area per molecule by ~ 15%. Ca binding also results in the formation of trans PS-Ca-PS complexes with melting temperatures of ~ 120º C for DOPS; these were characterized by NMR and X-ray diffraction.[7-14] More recently, high-resolution combined SAXS/SANS study of POPS in the Ca-free form from the group of Katsaras[15] and the study of PS in the absence of salt by the group of Nagle.[16]

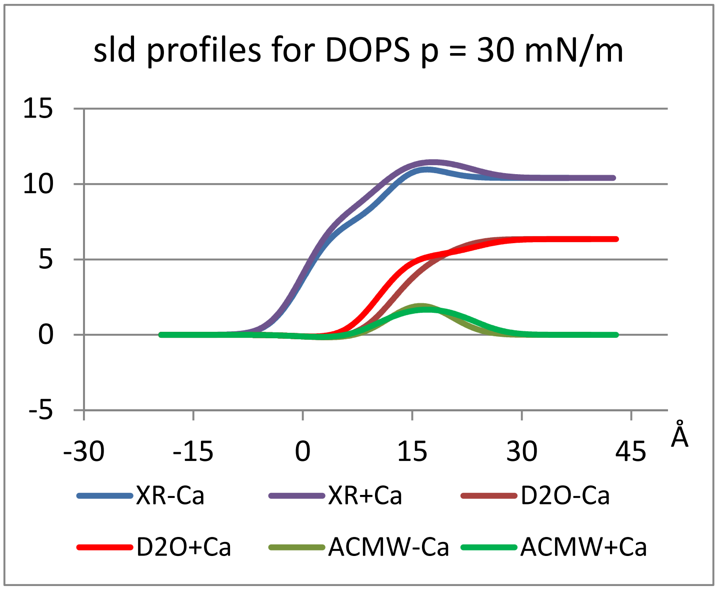

Figure 1: sld profiles generated from the fit to the XR and NR data for DOPS spread on +Ca or –Ca subphases. Two contrasts were used for NR: D2O and ACMW. A clear difference in the sld profiles in the presence and in the absence of Ca is observed.

	-Ca		**+Ca**	
	NR	**XR**	**NR**	**XR**
t1, Å	12.2	12.2	10.4	10.4
SLD1×10^{-6} Å^{-2}	-0.22	7.40	-0.22	8.01
solvent1,%	0.0	0.0	0.0	0.0
r1, Å	3.6	3.6	3.6	3.6
t2, Å	7.8	4.8	13.2	12.7
SLD2×10^{-6} Å^{-2}	3.92	12.44	4.00	11.61
solvent2,%	31	0	55	0
r2, Å	3.6	3.6	3.6	3.6
APM heads, Å^2	66	66		
Vhead, Å^3	514	321		

Table 1: Fitting parameters used to generate the SLDs shown in Figure 1. In the absence of Ca, layer 1 corresponds to the chains and layer 2 to the headgroups. Chain SLD (-0.22) and roughness (r) were held constant. Published molecular volumes were used to calculate APMs. In the presence of Ca, the interpretation of the two layers is more difficult due to the conformational change that the lipid undergoes.

Studying Ca-bound form of PS using multilayers or vesicles in solution is limited due to the formation of the high-melting phases. We circumvent this problem by performing neutron and X-ray reflectometry measurements (NR and XR) on PS monolayers at the air-water interface in the presence and in the absence of calcium. Within the context of this proposal, DOPS, deuterated POPS and hydrogenated POPS were studied on two contrasts each (D2O and air contrast matched water, ACMW). Subphases contained 10 mM CaCl2 or 10 mM EDTA. The data for DOPS were co-refined with the XR data that Tom Arnlold (Diamond, UK) has collected for us. XR data collection on POPS is pending. The co-refinement was done in MOTOFIT using a two-layer model (headgroups/tails, constrained to give the area per headgroup of DOPS ~ 68 Å^2/molecule in the absence of calcium[14] The results for DOPS are shown below for one surface pressure, 30 mN/m (Figure 1). There is a clear difference in the SLD profiles between the Ca-free and Ca-bound forms. The fitting parameters are shown in Table 1.

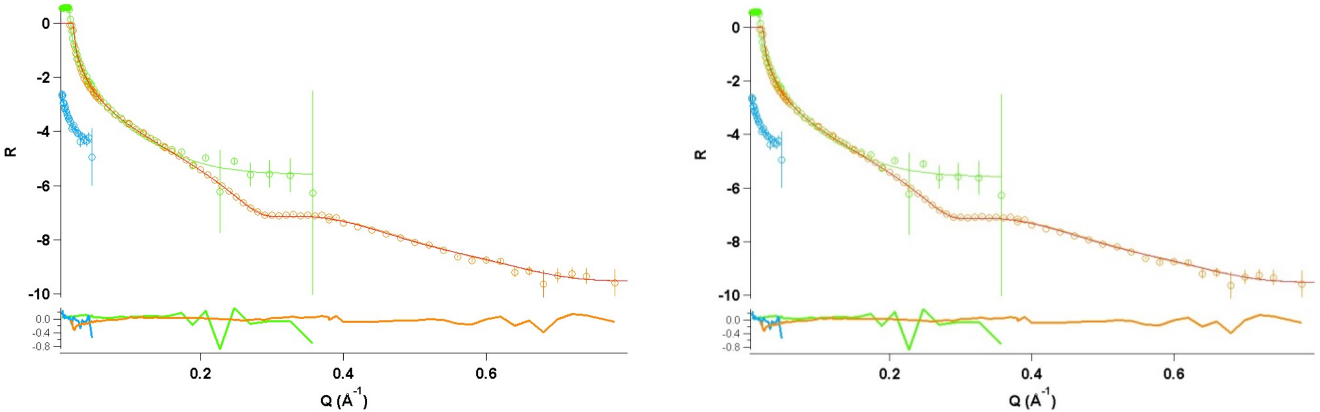

Figure 2: Reflectivity curves for DOPS monolayers spread at the air-water interface on the Ca-free (left) and Ca-containing (right) subphase. Both the neutron (green, blue) and the X-ray (orange) data are plotted. Lines are fits to the data with the two-layer model described in **Table 1**.

Conclusions: we performed all of the experiments we planned: two contrasts for DOPS, three contrasts for POPS (D2O and ACMW for dPOPS; ACMW for hPOPS), on Ca-free and Ca-containing subphases, at five different pressures: 10, 15, 20, 25, and 30 mN/m. We were able to jointly refine the neutron and the X-ray data for DOPS at 30 mN/m obtaining good fits and reasonable SLD profiles. Most importantly, we observed the effects of Ca binding to the PS headgroup. As far as we are aware of, this is the first such observation.

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