Experimental report

15/05/2019

Proposal: 9-13-733 **Council:** 4/2017

Title: Antimicrobial peptide loaded nanogels and their interaction with lipid bilayers

Research area: Soft condensed matter

This proposal is a resubmission of 8-02-793

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Samples: Lipid
C42H82NO8P
C27H46O
POPC, PAPC, Si

Instrument	Requested days	Allocated days	From	To
FIGARO	3	0		
D17	3	2	13/06/2018	15/06/2018

Abstract:

Due to increasing antibiotic resistance, antimicrobial peptides (AMPs) are of increasing interest as alternatives to classical antibiotics. Usually, AMPs are bactericidal via disruption of the bacterial membrane. However, AMPs tend to be degraded by the chemical and enzymatic environment in vivo, it is therefore important to protect the AMPs in drug delivery systems (DDS) to ensure effect after administration. Nanogels, designed to be biocompatible, provide a water-rich environment where the AMP can maintain its function, giving a suitable DDS for AMPs.
The mechanisms of action of DDSs are important to map. Ellipsometry data indicates an interesting synergistic effect of our proposed nanogel and AMP in the formulation. Initial reflectometry data shows that it is possible to see a difference between a bacteria mimicking PC:PG bilayer before and after introduction of AMP. Employing further contrasts should be able to elucidate whether the peptide is located in the bilayer or is also removed upon washing. We aim to compare these results to AMP-loaded nanogels to measure if the disruption mechanism changes upon encapsulation.

Experimental report 9-13-733: Effects of oxidation on the physicochemical properties of polyunsaturated lipid membranes

The exposure of biological membranes to reactive oxygen species (ROS) is key for many pathological conditions, including inflammation, infection, or sepsis. ROS also modulate important signaling processes and produce markers for damaged tissue. One of the main mechanisms behind these effects is lipid peroxidation of cell membranes and tissues, which is well known to deeply affect membrane properties such as structure or stability by the production of a complex mixture of oxidized lipid products. Previous studies of membrane oxidation have focused on monounsaturated or saturated lipids; however, polyunsaturated fatty acids (PUFA) are much more susceptible to oxidation as they contain several methylene groups located between double bonds, characterized by weaker C-H bonds and so more prompt to H extraction. Therefore, the inclusion of polyunsaturated phospholipids into membrane oxidation models seems crucial for modelling, characterizing, and understanding the complex processes occurring in real mammalian membranes and tissues.

The aim of this experiment was to investigate the effects of oxidative stress, originated by shortwave UV exposure, on phospholipid bilayers with different PUFA content, by following the structural changes during and after oxidation by neutron reflectometry (NR). These experiments were also correlated with other data obtained by complementary techniques, such as small-angle X-ray scattering (SAXS), Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR), fluorescence leakage assays, and light scattering, and all this work constituted the paper recently published by Parra-Ortiz et. al [1]

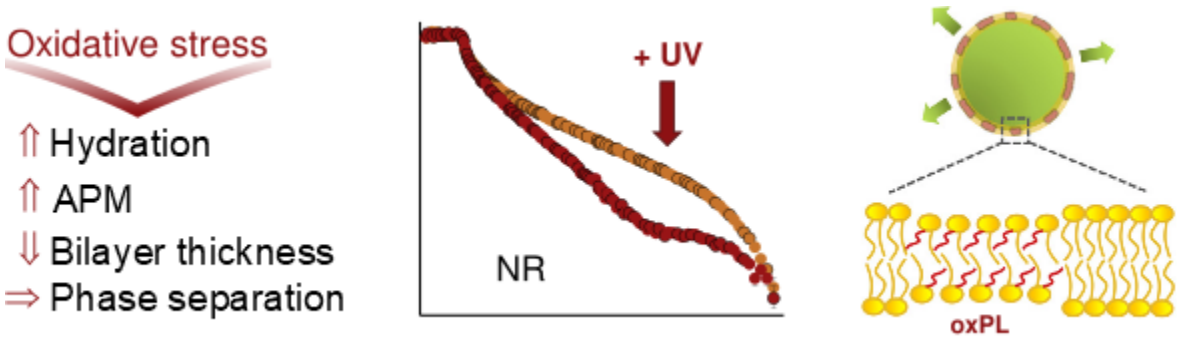

Figure 1. Summary of the performed experiments and main findings included in Parra-Ortiz et al [1].

In order to achieve the experimental aims, we used UV-transparent quartz substrates (80x50x15 cm) mounted on solid/liquid interface cells, whose top plates were modified with a central opening (3 cm diameter) to enable *in situ* UV illumination of the bilayers. The top-plates were kindly provided by Giovanna Fragneto. All cells were thermostatized at 37°C by using a circulating water bath.

Mixtures of the unsaturated phospholipids 1-palmitoyl-2-oleoyl-snglycero-3-phosphocholine (16:0–18:1 PC, POPC) and the polyunsaturated 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0–22:4 PC, PAPC) were used in this study. After bare surface characterization in two contrasts, H2O and D2O, bilayers of POPC:PAPC (0:100 or 50:50,

mol:mol) were prepared by vesicle deposition and fusion onto the UV-transparent quartz-blocks. Good bilayer coverages, and total thickness and area per molecule (APM) consistent with the literature, were obtained (see Table 1).

Table1. Fitting parameters used and obtained for the un-oxidized lipid bilayers of either 50:50 or 0:100 POPC:PAPC including SLD-values, thickness of the modelled layers, volume fraction (φ=1-hydration) and area per molecule (APM).

50:50 POPC:PAPC	SLD (10^{-6} Å^{-2})	Thickness (±1Å)	φ (±1%)	APM (±1Å^2)
Quartz	4.18	-	-	-
Heads	1.82	8.0	0.63	65.5
Tails	-0.185	26.8	0.99	66.0
0:100 POPC:PAPC	SLD (10^{-6} Å^{-2})	Thickness (±1Å)	φ (±1%)	APM (±1Å^2)
Quartz	4.18	-	-	-
Heads	1.82	8.0	0.58	72.0
Tails	-0.065	25.5	0.99	71.5

The lipid bilayers were characterized in three contrasts, hTris, dTris (10 mM Tris 150 mM NaCl, pH/pD 7.4) and a hTris:dTris mixture contrast matched to crystalline quartz (69:31 v/v, CMQTris), , and subsequently exposed to two different kinds of oxidation treatments: (i) 12 h incubation (37 °C) as autooxidation control in the absence of UV; or (ii) 2h of *in situ* UV exposure (37 °C). During UV exposure, time-resolved measurements were performed every minute to record the changes at an intermediate angle, 1.8°, covering the Q-region where the largest reflectivity changes were observed, by using divergent beam geometry, which improves statistics and allows for shorter acquisition times [2]. After the oxidation treatments, the lipid bilayers were fully characterized in dTris, and then rinsed and fully characterized again in the 3 contrasts. All contrast exchanges were done by pumping 20 mL of the desired contrast at 2 mL/min. The results for the two bilayer compositions tested before and after each oxidation treatment, only showing the d-contrasts for clarity, are presented in Figure 2. Some reflectivity changes were measured after autooxidation, especially for the pure PAPC bilayer (Figure 2B), but the changes were more important after UV exposure. These curves were fitted, the SLD profiles were calculated, and from them key physical parameters of the bilayers were obtained, including total thickness and hydration of the head and tail regions. From these parameters, total surface coverage and APM were calculated based on literature values of the molecular volumes. Relative changes of all these parameters with respect to the initial bilayers for each of the compositions and oxidation treatments are shown in Table 2.

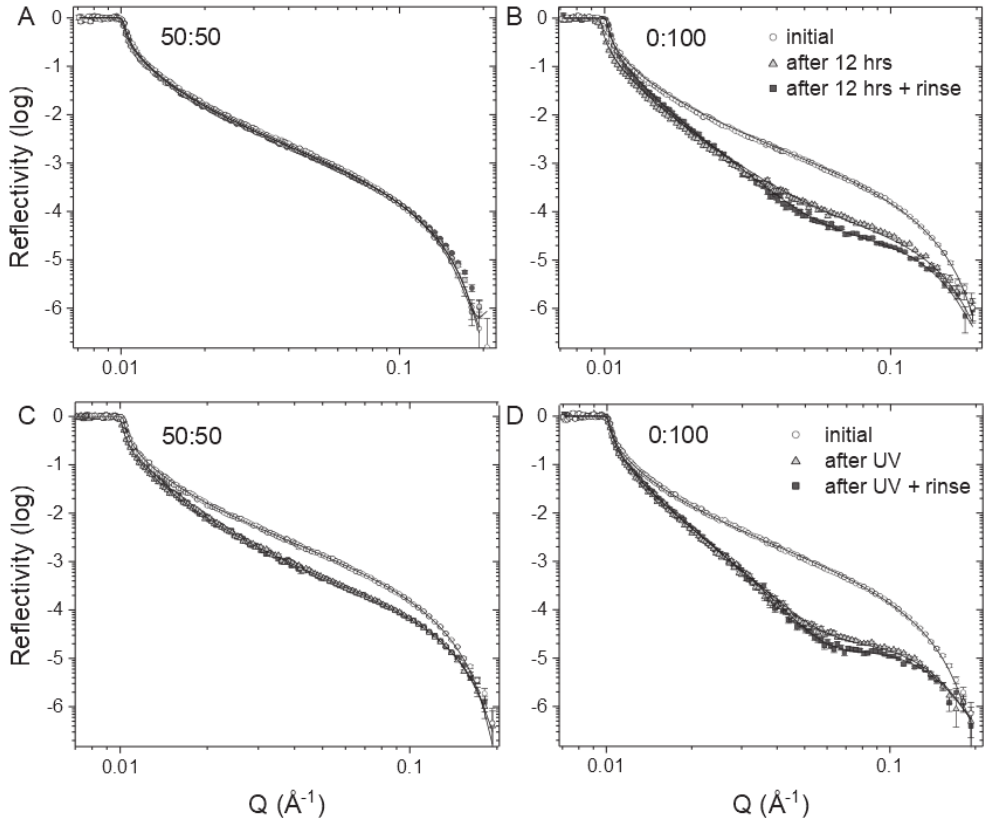

Figure 2. Neutron reflectivity profiles of POPC:PAPC bilayers in d-Tris including experimental data(markers) and fits (lines) subjected to either (A,B) 12 h incubation at 37 °C or (C,D) 2h UV-exposure.

Table 2. Changes in key physical parameters upon oxidation of POPC:PAPC (50:50 or 0:100) bilayers obtained by fitting the NR profiles, either after incubation at 37°C or after UV treatment. The parameters are presented as percentage of the values for the initial, unoxidized bilayers.

Parameter variation (%)	**12h incubation**		**2h UV exposure**	
	50:50	**0:100**	**50:50**	**0:100**
Bilayer thickness	0	0	-1	-5
Head hydration	-6	+58	+34	+66
Tail hydration	+4	+58	+33	+64
Surface coverage	-5	-58	-36	-67
APM	+5	+139	+53	+203

To summarize: The structural changes caused by oxidation of polyunsaturated POPC:PAPC lipid bilayers were studied by NR, both by 12 hrs incubation at physiological temperature and by 2 hrs of *in situ* UV exposure. It was found that bilayer autooxidation, but much more importantly UV exposure, increased tail and head group hydration and APM, translated into decreased surface coverage. . The composition of the bilayer was also found to be important, being these structural effects of oxidation much more pronounced for higher degrees of polyunsaturation present in the bilayer.

1. Parra-Ortiz, E., et al., *Effects of oxidation on the physicochemical properties of polyunsaturated lipid membranes.* Journal of colloid and interface science, 2019. **538**: p. 404-419 DOI: 10.1016/j.jcis.2018.12.007.
2. Cubitt, R., et al., *An improved algorithm for reducing reflectometry data involving divergent beams or non-flat samples.* Journal of Applied Crystallography, 2015. **48**(6): p. 2006-2011 DOI: 10.1107/S1600576715019500.