Experimental Report

Proposal: **9-10-1241** **Council:** 4/2012

Title: Bilayer oxidation using labelled POPC to confirm chemical mechanism and structure of decay by ozone oxidation.

This proposal is a new proposal

Researh Area: Chemistry

Main proposer: **KING Martin**

Experimental Team: JONES Stephanie
RENNIE Adrian R.
KING Martin

Local Contact: WATKINS Erik

Samples: 1-Palmitoyl-2-(d18)-oleoylphosphatidylcholine (C42H64D18NO8P)
1-palmitoyl(d31)-2-oleoyl-sn-glycero-3-phosphocholine (C42H51NO8PD31)

Instrument	Req. Days	All. Days	From	To
D17	3	3	26/10/2012	29/10/2012

Abstract:

This proposal follows a very successful proof-of-concept study and we request time to elucidate the mechanism for the chemically more complex POPC.

Oxidation of membrane lipids in biology is important because it may be a mechanism for ageing, cell apoptosis and cancer. We propose to use our skills obtained in oxidation of fatty acids and lipids at the air-water interface to investigate the oxidation of a bilayer at solid-water interface. Our oxidant (ROS) will be aqueous ozone and our bilayer will be two different isotopologues of POPC: half-deuterated oleyl chain, or fully deuterated palmitoyl chain supported on a quartz window in a flow cell. Half-deuterated Oleyl chain POPC has recently been synthesised. Neutron reflection will allow us (1) to record the kinetic decay of head only, tail only and complete lipid by selective deuteration (2) investigate the change in morphology (film thickness and integrity) of the oxidized lipid bilayer kinetically. The bilayer, oxidant, analysis and technique are either well- studied systems or systems we have used previously. We will provide kinetics, chemical mechanism and morphological data on the oxidation of a biological relevant bilayer.

Experimental report No. 9-10-1241: Bilayer oxidation using labelled POPC to confirm chemical mechanism and structure of decay by ozone oxidation.

M.D. King, K.C. Thompson, A.R. Rennie, and R.A. Campbell.

This was a difficult experiment that suffers numerous technical issues. However, some very useful information was collected that can be written up as a peer-review article.

Introduction and background

Oxidation of membrane lipids in biology is a very important field because it may impact ageing,[1] cell apoptosis[2] and cancer[3]. It is unclear what the chemical identity of the oxidant is and there is plenty of discussion in the literature. Consequently the term ROS (reactive oxygen species) is invoked and that may include OH, $O_2(^1\Delta_g)$, HO_2, O_2^- etc. Studies of the oxidation of lipid bilayers (as a proxy for biological membranes), generate mixtures and unknown amounts of ROS and describe typically what happens to either the morphology of the bilayer/lipid (e.g [4]) or report the resultant products (e.g [5]). We wish to use our skills of chemical mechanism and chemical oxidation gained on the oxidation of amphiles (lipids and insoluble monolayers)at the air-water interface[6] to study the oxidation of lipid bilayers. We generate known amounts of a single ROS species and conduct experiments that will simultaneously (a) highlight the location on the lipid molecule of initial attack (head, tail or both?), (b) determine the site specific rate constants of the bilayer attack and (c) in real time record the change in bilayer morphology (film thickness) by neutron reflection. We will start with a well-behaved lipid bilayer, DPPC that can be part or fully deuterated and a radical from the ROS family, the hydroxyl radical.

Experimental

We used a well established vesicle fusion mechanism to place a bilayer of deuterated DPPC at the solid-liquid interface of a quartz-water cell.[7] The cell was a sandwich of a quartz windows and a plexiglass window separated by a PTFE spacer. A quartz contrast matched aqueous solution of 1% (m/m) hydrogen peroxide was gently flowed through the cell. The solution was illuminated with UV light (wavelength of 365 nm) through the silica window. Figure 1 has a picture of set-up on D17. The photolyis generated HO_2 and OH radicals in aqueous solution in known quantities from Haber-Weiss. We ran two contrasts with quartz matched aqueous solution of hydrogen peroxide and a pure H_2O aqueous solution of hydrogen peroxide. With careful modelling of the neutron scattering as a function of momentum transfer, the loss of neutron reflection was attributed to the loss of specific (deuterated) regions of the lipid molecule.

Preliminary results

There was some successful experiments undertaken but there were numerous technical difficulties. Figure 2 demonstrates the surface coverage of the DPPC bilayer (as determined by neutron reflection) as a function of time during oxidation by OH radicals. The figure demonstrates the raw data from that experiment. The surface coverage shows a gain of scattering length from increased oxygen in the surface layer and then a subsequent loss of deuterated material with time. There is a reorganization of surface followed by an almost exponential decay and almost complete removal of the bilayer. The film thickness also changed, providing tentative evidence of hydroxyl radicals entering the bilayer and oxidizing the tails.

References

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Figure 1
The Solid-liquid cell mounted on the D17 beamline before (LHS) and after (RHS) addition of the UV photolysis lamp.

Figure 2
Preliminary results from the experiment. The surface coverage of the lipid molecules are plotted as a function of time, as determined from neutron reflection. Tentatively we can suggest there appears to be a period of re-arrangement followed by complete oxidation and loss of nearly all material from the interface.