Experimental report

10/02/2017

Proposal: 8-02-720 **Council:** 10/2014

Title: Neutron diffraction studies of membrane rafts

Research area: Biology

This proposal is a new proposal

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Samples: cholesterol
dipalmitoylphosphatidylcholine
dioleoylphosphatidylcholine

Instrument	Requested days	Allocated days	From	To
D16	7	7	20/07/2015	27/07/2015

Abstract:

The lateral organisation of lipids and proteins in the eukaryotic cell membrane has been the focus of much recent research. Of particular interest is how some lipid components of the membrane can phase separate to form highly dynamic, nanoscale structures known as lipid rafts. The present study uses neutron diffraction to study the effect of temperature and composition on the formation of rafts prepared using dioleoylphosphatidylcholine (DOPC), cholesterol (chol) and dipalmitoylphosphatidylcholine (DPPC). Significantly these studies will enable the thickness of the different domains present in the raft to be determined; information that cannot be obtained using other techniques available to study rafts. These studies will complement other neutron scattering studies and enable us to build up a detailed picture of molecular architecture of rafts in bilayer membranes.

8-02-720 Neutron Diffraction Studies of Membrane Rafts

Background The lateral organisation of lipids and proteins in the cytoplasmic membrane of eukaryotic cells has – and continues to be – the focus of intense research. Of special interest is how the lipid components of the membrane can be triggered to phase-separate and form highly dynamic, nanoscale structures called 'rafts' (1). Indeed, it is well established that under physiological conditions a membrane composed of phospholipid (PL), cholesterol (Chol) and sphingolipid (SL) can be triggered to phase-separate forming distinct, liquid ordered (L_o) domains/rafts composed of Chol and the highly saturated, conformationally ordered SLs (2,3) "floating" within a bed of "liquid-like" unsaturated and conformationally disordered PLs in a liquid disordered (L_d) phase (4,5). Significantly, the association of several classes of membrane proteins with these lipid domains has led to the so-called ''raft hypothesis'' wherein rafts are seen to be key in a variety biological processes such as cell signalling and membrane trafficking (6).

Introduction to the Proposal The phenomenon of membrane lipid raft formation has to date been investigated primarily in a qualitative manner using fluorescence techniques, with a small number of (quantitative) X-ray diffraction studies performed to study raft formation in lipid vesicles (cf., 6). These experiments have generally been performed to look at the effects of changing sample composition and temperature, and have involved samples prepared from ternary lipid mixtures, typically palmitoyloleoyl- or dioleoyl-phosphatidylcholine (POPC, DOPC), mixed with dipalmitoylphosphatidylcholine (DPPC) and cholesterol (Chol) (7-10).

In specular and off-specular neutron reflectivity (NR) studies of DPPC:DOPC:Chol and DPPC:POPC:Chol monolayers performed using OFF-SPEC at ISIS, we recently established that while the thickness and composition of the lipid monolayer remain constant with changing temperature, over the range 10 ^{o}C – 25 ^{o}C, there is significant off-specular scattering seen at 10 ^{o}C for the monolayers that comprise a 2:2:1 molar ratio of DPPC:DOPC/POPC:Chol.

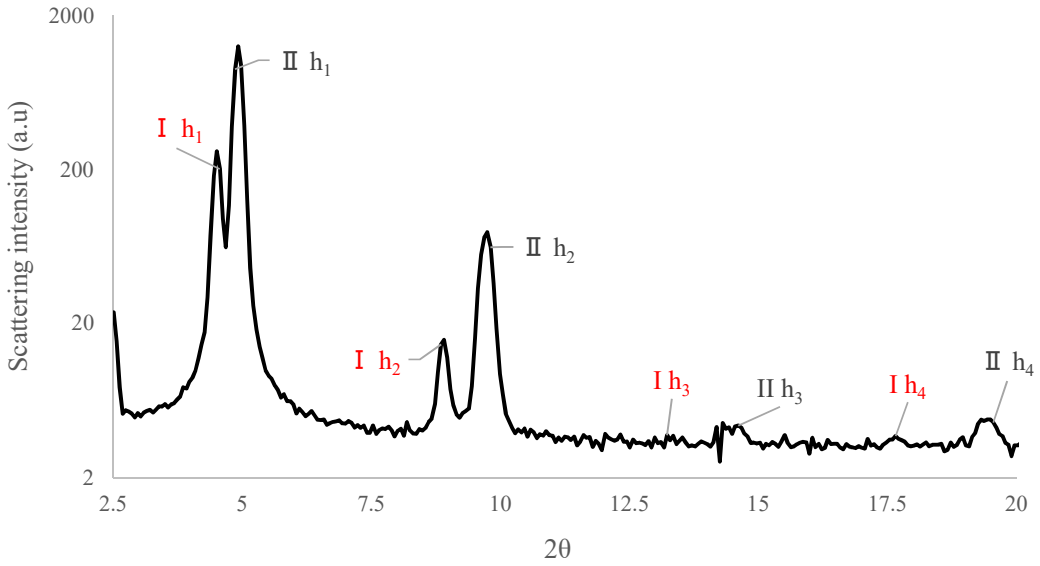
In the associated SANS experiments which we performed (on Sans-2d at ISIS) using small unilamellar vesicles, we subsequently studied the formation of rafts as a function of lipid composition and temperature, and showed that while raft formation was complete at 15 ^{o}C for vesicles composed of a 2:2:1 DPPC:DOPC:Chol system, it was incomplete at 10 ^{o}C for vesicles prepared from a 1:1:1 DPPC:DOPC:Chol mixture. In agreement with our NR measurements, the 'off contrast' SANS results for these systems showed that despite the raft formation as indicated by the off-specular scattering, the thickness of the corresponding vesicle bilayers remained unchanged with changing temperature – this likely being a consequence of the rotational averaging arising from the vesicle motion within the preparation.

Aim of the Experiment In 8-02-720 we aimed to carry out neutron diffraction studies on multilayers comprising 2:2:1 ternary lipid mixtures of DPPC:DOPC:Chol, (1) to determine the effects of temperature on raft formation, (2) to determine the *d*-spacing and (3) to determine (the scattering length density profiles of the systems *via* Fourier reconstruction using the calculated structure factors for the various phases).

Sample preparation Samples were prepared by dissolving ~10 mg of each DPPC/DOPC/Chol mixture in 2 ml chloroform/methanol (2:1, v/v), and multilayers then prepared on 3 cm diameter silicon wafers. Diffraction measurements were performed with detector distances arranged to cover a Q range of 0.03 Å^{-1} and 0.4 Å^{-1}. Structure factor phases were determined using the water distribution – employing samples of varying contrast, hydrated under 3 different H_2O/D_2O atmospheres. Changes of sample hydration wiere performed in a thermostatically regulated humidity chamber, allowing ~12 h for (off-line) annealing (at 60^{o}C). Each system was studied at a relative humidity (RH) of 100% and 80%, and measurements made at 25 ^{o}C, 20 ^{o}C, and 15 ^{o}C.

Results Diffraction patterns were successfully recorded for the lipid multilayers at 25 ^{o}C, 20 ^{o}C, and at 15 ^{o}C, with RH of 100% and 80%. The attempts made to record measurements at 10 ^{o}C proved highly problematic because of excessive condensation caused by the high ambient temperature in the experimental hall. Further problems were also encountered arising from a malfunctioning thermistor/humidity sensor.

a)



b)

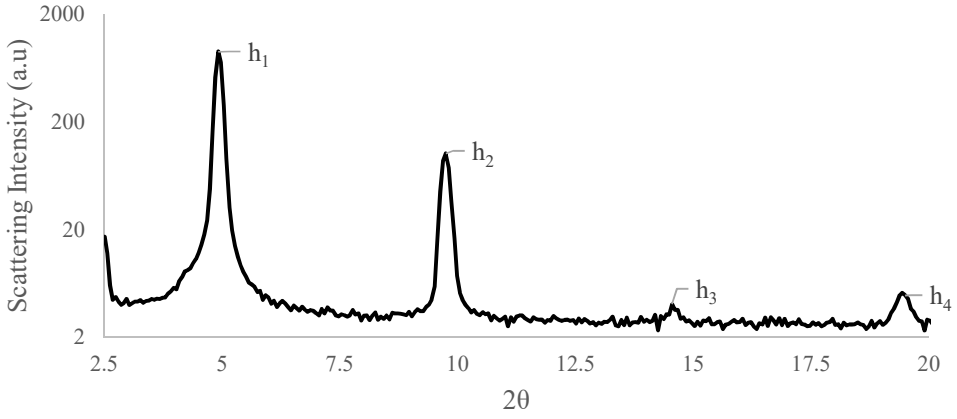


Figure 1 Diffraction patterns and the corresponding Bragg peaks for *h*-DPPC/*h*-DOPC/*h*-Chol bilayer stack recorded at 15 °C (a) and 25 °C (b) both at 80% RH and in 100% D_2O. A single phase is seen at 25 °C, and two phases at 15 °C (phases I and II).

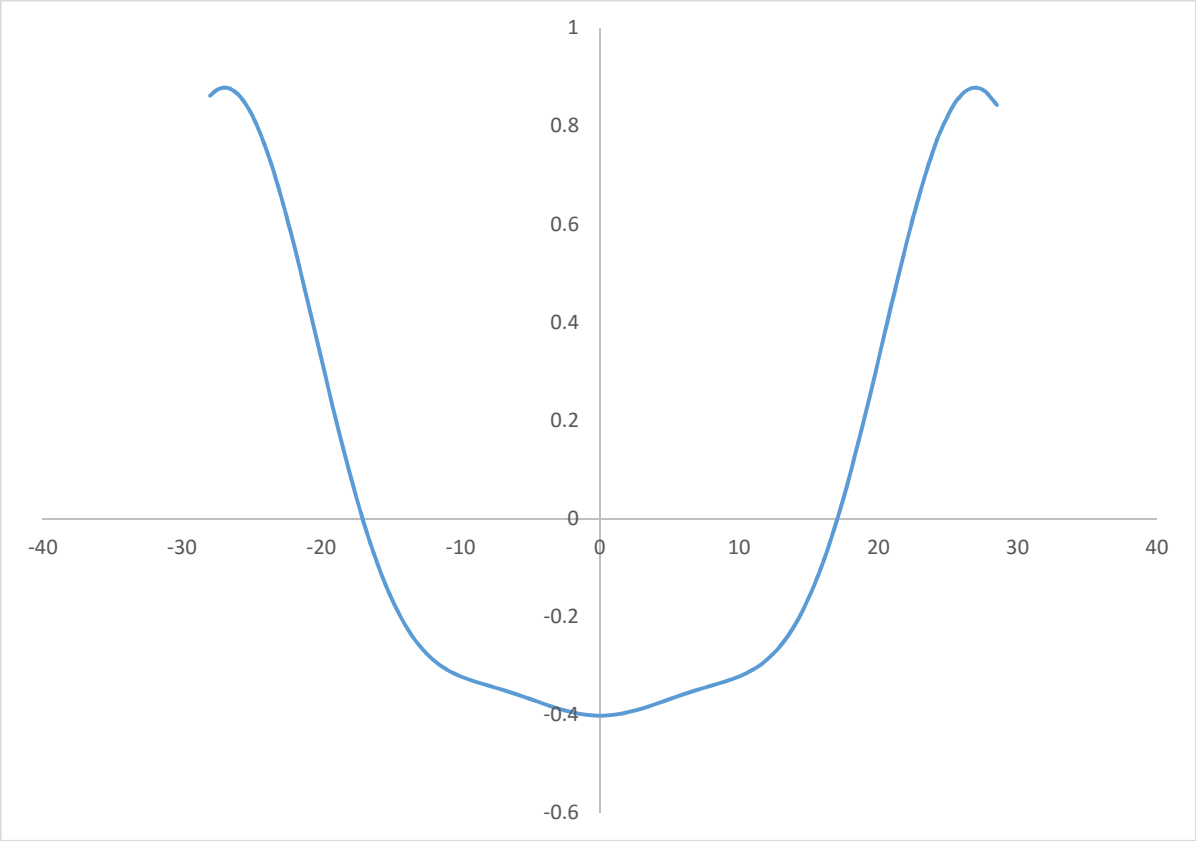


Figure 2 Scattering length density profile for the 2:2:1 DOPC:DPPC:Chol multilayers calculated with structure factors determined for the sample maintained at 80% RH and 15°C (*d*-spacing = 53.9 Å)

Notwithstanding these issues, we were successful in making sufficient measurements to demonstrate the presence of a single lipid phase existing at 25 °C, with a *d*-spacing of 53.8 Å; and two phases existing at 15 °C, with *d*-spacings of 53.9 Å and 59.2 Å (see Figure 1). The necessary structure factor amplitudes and phases were determined for each of these systems, and the corresponding scattering length density profiles then constructed (*cf.*, Figure 2).

Given that all experiments were performed using only protiated lipid samples, however, we were unable to determine (in an unambiguous manner) the internal architecture of the different systems.

References

(1) Longo & Blanchette (2010) Biochim Biophys Acta 1798:1357. (2) Simons and Ikonen (1997) Nature 387:569. (3) Fanani *et al* (2010) Chem Phys Lipids 163:594. (4) Brown (1998) J Cell Sci 111:1. (5) London (2002) Cur Opin Struct Biol 12:480. (6) Quinn (2010) Progr. Lipid Res. 49:390. (7) Bacia *et al* (2004) Biophys J 87:1034. (8) de Almeida *et al* (2003) Biophys J 85:2406. (9) de Almeida *et al* (2005) J Mol Biol 246:1109. (10) Keller *et al* (2000) Biophys J 79:2033: