Journal of Colloid and Interface Science 457 (2015) 225–231

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

The interactions of squalene, alkanes and other mineral oils with model membranes; effects on membrane heterogeneity and function

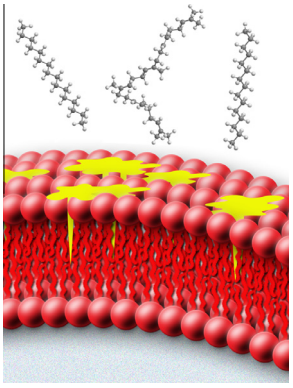
Joanna L. Richens, Jordan S. Lane, Melissa L. Mather, Paul O'Shea *

Cell Biophysics Group, Institute of Biophysics, Imaging and Optical Science, School of Life Sciences, University of Nottingham, University Park, Nottingham, United Kingdom

HIGHLIGHTS

- Oil–phospholipid interactions modify the membrane dipole potential.
- Residual oil has no significant effect on fatty acid movement across the bilayer.
- Residual oil leads to increased frequency of membrane microdomains.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 April 2015
Revised 18 June 2015
Accepted 18 June 2015
Available online 11 July 2015

Keywords:
Droplet interface bilayers
Mineral oil
Alkanes
Squalene
Membrane microdomains

ABSTRACT

Droplet interface bilayers (DIBs) offer many favourable facets as an artificial membrane system but the influence of any residual oil that remains in the bilayer following preparation is ill-defined. In this study the fluorescent membrane probes di-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate (Di-8-ANEPPS) and Fluoresceinphosphatidylethanolamine (FPE) were used to help understand the nature of the phospholipid–oil interaction and to examine any structural and functional consequences of such interactions on membrane bilayer properties. Concentration-dependent modifications of the membrane dipole potential were found to occur in phospholipid vesicles exposed to a variety of different oils. Incorporation of oil into the lipid bilayer was shown to have no significant effect on the movement of fatty acids across the lipid bilayer. Changes in membrane heterogeneity were, however, demonstrated with increased microdomain formation being visible in the bilayer following exposure to mineral oil, pentadecane and squalene. As it is important that artificial systems provide an accurate representation of the membrane environment, careful consideration should be taken prior to the application of DIBs in studies of membrane structure and organisation.

Abbreviations: SUV, small unilamellar vesicles; GUV, giant unilamellar vesicles; DIB, droplet interface bilayers; Di-8-ANEPPS, di-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate; FPE, Fluoresceinphosphatidylethanolamine; DPhPC, 1,2-diphytanoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; PC, phosphatidylcholine; PLV, phospholipid vesicles.

* Corresponding author at: Cell Biophysics Group, Institute of Biophysics, Imaging and Optical Science (IBIOS), School of Life Sciences, University of Nottingham, Nottingham, England NG7 2RD, United Kingdom.

E-mail addresses: joanna.richens@nottingham.ac.uk (J.L. Richens), jordan.lane@nottingham.ac.uk (J.S. Lane), melissa.mather@nottingham.ac.uk (M.L. Mather), paul.oshea@nottingham.ac.uk (P. O'Shea).

http://dx.doi.org/10.1016/j.jcis.2015.06.052
0021-9797/

1. Introduction

Biological membranes comprise a complex mixture of lipids, proteins and carbohydrates, unique for each cell type, which contribute to efficient cellular function. The complexity of these entities makes it difficult, however, to use whole-cell systems to examine the contribution of individual components to any given cellular process. Artificial membrane systems including small unilamellar vesicles (SUVs; 10's of nanometers), giant unilamellar vesicles (GUVs; 10's of microns), supported bilayers, natural cell membrane fragments and computer simulations [1] are, therefore, crucial to our understanding of cellular function, providing tools through which structural and functional properties of the lipidic components of cell membranes can be investigated [2]. Droplet interface bilayers (DIBs) are relatively new within the field having only been developed over the last decade [3]. In the presence of phospholipids, a monolayer will form at an aqueous–oil interface; DIB formation works on the principle that bringing two aqueous droplets into close proximity in an oil environment will result in the formation of a lipid bilayer at the point of contact between the individual monolayers at the two droplet interfaces [4]. This technique can be achieved by inclusion of the phospholipids in either the aqueous ('lipid-in') or oil ('lipid-out') phase [5].

DIBs are an increasingly popular option as a model membrane system as they offer many favourable properties including the potential to dictate size, curvature and asymmetry of the bilayer and have high levels of durability [6,7]. They have already been utilised in studies examining, for example, ion channel transport [6,8], and as technologies associated with DIB production and characterisation continue to evolve so too will the potential and scope of downstream applications [9–12]. Any model system must, however, provide a true representation of the membrane environment under consideration. A variety of oils have been used during the generation of DIBs including different alkanes [3,4,12,13], mineral oil [14], squalene [3] and soybean oil [15] and whilst the propensity of some of this oil to remain within the bilayer has been demonstrated previously, the extent and consequences of these interactions has not been fully elucidated and appear to be dependent upon the specific oil used within the protocol. In one case, for example, it was calculated that 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) DIBs would contain 9.2% hexadecane (used in many DIB preparation protocols [3,12]) or 38% decane by volume [16]. This is in comparison to DIBs formed in cis-9-tricosene which produced a lipid bilayer which was essentially solvent-free [16]. It is imperative to future applications of DIB technologies that the implications of any residual oil remaining in the bilayer following DIB production are better characterised. In this study the fluorescent membrane probes D i-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate (Di-8-ANEPPS) and Fluoresceinphosphatidylethanolamine (FPE) are used to help understand the nature of the phospholipid–oil interaction and to examine any consequences that these interactions have on membrane properties and functions including movement of fatty acids across the lipid bilayer and membrane patterning or heterogeneity.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC; 850355C) was purchased from Avanti Polar Lipids (Alabama, USA). Egg phosphatidylcholine (PC) was supplied by Lipid Products (UK). Di-8-ANEPPS (D-3167) and FPE (F-362) were purchased from Life Technologies (Paisley, UK). Cholesterol (Chol) (C8667), hexadecane (H6703), mineral oil (M5904), oleic acid (OA) (O1008), pentadecane (P3406), squalene (S3626) and all other reagents were supplied at the highest purity available by Sigma Aldrich (Poole, UK).

2.2. Oil-in-water emulsion concentration determination

Working concentrations of oil in water emulsions were determined using methods previously developed for critical micelle concentration determination [17]. Briefly, hexadecane, mineral oil, pentadecane and squalene were diluted in the appropriate solvent (e.g. ethanol) until addition of 0.5% v/v to 10 mM tris buffer gave solutions of the desired concentrations. Samples were incubated at 25 °C, protected from light, for 30 min after which time the light scattering at 90° was monitored with a Fluoromax-4 Spectrofluorimeter (HORIBA Jobin Yvon), using a 600 nm irradiation wavelength and a 2 nm bandpass. Data obtained were normalised to controls containing equivalent volumes of buffer and solvent. Changes in light scattering intensity were plotted against sample concentration and a one sample *t*-test was employed at each concentration ($n = 3$) to determine whether the scattering intensity significantly deviated from background scatter. When scattering intensity deviated significantly, a second linear equation was fitted to the dataset, the intercept of the two models was reported, and concentrations above and below this value were selected for use in further experiments.

2.3. Phospholipid vesicle preparation

Phospholipid vesicles (PLVs) were prepared as previously described [18,19]. Briefly, the desired phospholipid and cholesterol dissolved in chloroform–methanol (ratio 5:1) were mixed in a round bottom flask and dried under a stream of oxygen-free nitrogen gas by rotary evaporation until a thin film was formed. The lipid film was rehydrated with 10 mM tris buffer pH 7.4 to a final total concentration of 13 mM. The resulting multilamellar solution was frozen and thawed 5 times and extruded 10 times through 25 mm diameter polycarbonate filters with pores of 100 nm in diameter (Nucleopore Corp.) using an extruder (Lipex Biomembranes Inc.) according to previously described extrusion procedures [20]. This resulted in a monodisperse, unilamellar suspension of PLVs.

2.4. Phospholipid vesicle labelling with fluorescent probes (FPE & Di-8-ANEPPS)

PLVs were labelled in the outer bilayer leaflet with FPE as previously described [19]. Briefly, the PLVs were incubated with ethanolic-FPE (never more than 0.1% v/v ethanol) at 37 °C for 1.5 h in the dark. Unincorporated FPE was removed by gel filtration on a PD10 Sephadex column. For the labelling of phospholipid vesicles with Di-8-ANNEPS the phospholipid vesicles were incubated for at least 1 h at 37 °C in the dark in the presence of ethanolic-Di-8-ANNEPS (14.8 μg/ml).

2.5. Spectroscopy

Fluorescence spectroscopy of PLVs (400 μM) labelled with either FPE or Di-8-ANEPPS was conducted on a Fluoromax-4 Spectrofluorimeter (HORIBA Jobin Yvon). For FPE-based experiments, excitation and emission wavelengths were set at 490 and 518 nm respectively. Di-8-ANEPPS spectra were obtained by exciting the samples at 468 and 520 nm and measuring the emission ratio at 580 nm [21,22]. Fluorescence intensity was measured pre and post additions of appropriate concentrations of mineral oil (0.00005% and 0.001% v/v), hexadecane (0.5 μM and 8.5 μM), pentadecane (10 μM and 60 μM) or squalene (0.25 μM and 5 μM) to phospholipid vesicles (400 μM lipid) at 25 °C. Experimental