Chemistry and Physics of Lipids 165 (2012) 151–159

Contents lists available at SciVerse ScienceDirect

Chemistry and Physics of Lipids

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

Effects of cholesterol on phospholipid membranes: Inhibition of the interdigitated gel phase of F-DPPC and F-DPPC/DPPC

Eric A. Smith, Weidong Wang, Phoebe K. Dea*

Department of Chemistry, Occidental College, 1600 Campus Road, Los Angeles, CA 90041, USA

ARTICLE INFO

Article history:
Received 22 October 2011
Received in revised form 9 December 2011
Accepted 10 December 2011
Available online 16 December 2011

Keywords:
Interdigitated gel phase
Cholesterol
Fluorinated lipid
Fluorescence
X-ray diffraction
Differential scanning calorimetry

ABSTRACT

Unlike the parent phospholipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), the monofluorinated analog, 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine (F-DPPC), spontaneously forms an interdigitated gel phase (L_βI) below the main transition temperature (T_m). We have examined the effects of introducing cholesterol to F-DPPC and 1:1 F-DPPC/DPPC membranes using a combination of DSC, optical density, fluorescence intensity and polarization, ^{31}P NMR, and X-ray diffraction techniques. Cholesterol increases the fluidity of the gel phase, broadens the main transition, and decreases the main transition enthalpy. However, these results also reveal that there is an unusually large degree of phase coexistence between the L_βI and non-interdigitated gel phases when cholesterol is added. Cholesterol encourages this phase segregation by partitioning into the thicker non-interdigitated domains. At higher cholesterol concentrations, the majority or all of the L_βI phase of F-DPPC and 1:1 F-DPPC/DPPC is eliminated and is replaced by a non-interdigitated liquid-ordered (l_o) phase with properties similar to DPPC/cholesterol. Consequently, cholesterol mitigates the influence the C—F moiety has on the thermodynamic phase behavior of F-DPPC. Our findings demonstrate that there are multiple characteristics of cholesterol-rich membranes that disfavor interdigitation.

1. Introduction

The permeability, molecular order, elasticity, orientation and intermolecular spacing of lipid membranes are heavily dependent on cholesterol content (Corvera et al., 1992; Davis, 1993; Clarke et al., 2006; Asakawa and Fukuma, 2009). Cholesterol is also a major component of lipid rafts which are increasingly implicated in biological processes (Simons and Toomre, 2000; Binder et al., 2003). Biomolecules such as glycosylphosphatidylinositol-anchored proteins have been shown to preferentially associate with lipid rafts (Schroeder et al., 1998). The effectiveness of some synthetic anticancer alkylphospholipids that target cell membranes appear to be reliant on the presence of lipids rafts (Van der Luit et al., 2007). These alkylphospholipid analog therapies may also disrupt cholesterol homeostasis in cells and affect the composition of rafts (Heczková and Slotte, 2006). Additionally, the formation of caveolae, a special class of lipid domain in biological membranes, and caveolae-related endocytosis are reliant on the concentration of cholesterol (Chang et al., 1992; Binder et al., 2003). Due to the wide-ranging effects of cholesterol, it is essential to determine the nature of cholesterol–lipid interactions.

If fluorinated lipids are to be used in a biological environment, it is also important to understand how they behave in the presence of naturally occurring lipids such as DPPC and cholesterol. Fluorine-labeled lipids have been used to study the properties of proteins and membranes using ^{19}F NMR techniques (Post et al., 1981,1984). The lipid used in this study, F-DPPC, has been used as a rotational-echo double-resonance (REDOR) probe to analyze peptides integrated into phospholipid bilayers (Toke et al., 2004). Various fluorinated lipids also show promise as drug carriers (Dafik et al., 2009; Krafft and Riess, 2009).

Moreover, the L_βI phase has recently been shown to have an important application in the preparation of potential drug-encapsulating liposomes. The interdigitation-fusion procedure uses ethanol- or pressure-induced interdigitation to yield vesicles of high internal volume (Perkins et al., 1995; Ahl and Perkins, 2003). This process can be used to entrap smaller particles within a larger

Abbreviations: DHPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; β-DPH HPC, 2-(3-(diphenylhexatrienyl) propanoyl)-1-hexadecanoyl-*sn*-glycero-3 -phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPeth, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanol; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); DSC, differential scanning calorimetry; F-DPPC, 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine; L_βI, interdigitated gel phase; l_o, liquid-ordered phase; PVP, poly(vinylpyrrolidone); SAXS, small-angle X-ray scattering; T_m, main transition temperature; WAXS, wide-angle X-ray scattering.

* Corresponding author. Tel.: +1 323 259 2625; fax: +1 323 341 4912.
E-mail address: dea@oxy.edu (P.K. Dea).

0009-3084/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2011.12.006

Table 1
The effect of cholesterol on some previously studied interdigitated systems. The approximate mol% above which cholesterol has been reported to eliminate interdigitation is listed. Due to the limited number of concentrations tested, the values reported here may not reflect the absolute minimum amount of cholesterol required to prevent interdigitation.

Lipid	DPPC/EtOH[a]	DPPC/Pressure[b]	DPPeth/Tris–Cl[c]	DPPG/Tris–Cl[c]	DHPC[d]
Cholesterol	>20%	>8%	>10%	>20%	>5%

[a] 2.0 M EtOH; (Komatsu and Rowe, 1991).
[b] 145 MPa; (Tamai et al., 2008).
[c] 50 mM Tris–HCl, pH 7.4; (Bondar and Rowe, 1998).
[d] (Laggner et al., 1991; Cunningham et al., 1995).

surrounding vesicle to create a multi-compartment "vesosome" structure (Kisak et al., 2004). It is known that adding cholesterol hinders the effective creation of these liposomes, so it is necessary to identify the mechanism for why this occurs (Perkins et al., 1995). Additionally, incorporating fluorinated lipids such as F-DPPC could be used to encourage interdigitation during the required intermediate step.

F-DPPC is of particular interest because it spontaneously forms the L_βI phase below the T_m (Hirsh et al., 1998; Sanii et al., 2010; Smith et al., 2010). In the L_βI phase, the lipid hydrocarbon chains interpenetrate into the opposing monolayer, maximizing van der Waals forces and reducing head group crowding (Slater and Huang, 1988). This is usually accompanied by an increased exposure of the lipid hydrocarbon chains to the surrounding water. However, F-DPPC is unique because the highly polar C—F bond at the terminal end of the *sn*-2 acyl chain makes it more hydrophilic (Hirsh et al., 1998). The fluorine substitution on the *sn*-2 chain is even able to overcome the unfavorable interaction of the unmodified hydrophobic *sn*-1 chain with the aqueous solvent. This is thought to be possible because of the high conformational order and tight packing of the L_βI phase (Sanii et al., 2010).

Like F-DPPC, some phospholipids (e.g. the ether-linked DHPC) interdigitate when hydrated (Cunningham et al., 1995). However, most other lipids that can interdigitate (e.g. DPPC) only do so under specific chemical environments or with the application of hydrostatic pressure (Komatsu and Rowe, 1991; Tamai et al., 2008). In these lipid systems, cholesterol consistently inhibits the formation of the L_βI phase (Table 1). At intermediate concentrations of cholesterol, coexistence between interdigitated and non-interdigitated phases has been reported in systems such as DPPC/ethanol/cholesterol and DHPC/cholesterol (Laggner et al., 1991; Rosser et al., 1999; Tierney et al., 2005). The uneven packing and membrane width resulting between these domains can significantly alter membrane properties such as permeability and macromolecular shape (Komatsu and Okada, 1997). Since the previously studied lipid systems focus on lipids with highly hydrophobic hydrocarbon chains, we sought to determine if the interdigitated phase of the acyl chain modified F-DPPC is similarly disrupted by cholesterol.

2. Materials and methods

2.1. Materials

The water used in all samples was purified and double-deionized by a Milli-Q filtration system. The lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine (F-DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL). The fluorescent probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 2-(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine (β-DPH HPC) were obtained from Molecular Probes (Eugene, OR). Cholesterol (≥99%), methanol (≥99.9%), pharmaceutical grade poly(vinylpyrrolidone) (PVP, molecular weight 40,000), and Triton X-100 (Ultra molecular biology grade, ~10% assay in water) were obtained from Sigma–Aldrich (St. Louis, MO). Deuterium oxide (99.9%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). All purchased chemicals were used without further purification.

2.2. Methods

Samples for DSC, fluorescence, NMR, and X-ray diffraction experiments were prepared from combinations of stock solutions of lipid and cholesterol dissolved in methanol. All of the samples were evaporated to dryness followed by high vacuum for at least 10 h to remove any residual solvent. Each sample was then hydrated and incubated for 1 h at 60 °C with periodic vortexing.

2.2.1. Differential scanning calorimetry

All calorimetry samples contained ~3 mg lipid and were hydrated with 150 μl water. These were analyzed with a Calorimetry Sciences Corporation multi-cell DSC-HT Model 4100 DSC. Heating and cooling scans were recorded from 30 °C to 60 °C at a rate of 10 °C/h. The standard deviation of the T_m was ±0.1 °C. Enthalpy values were calculated from the calorimetry data using the integration function on OriginPro 7.5.

2.2.2. Optical density

To quantify Trition X-100 solubilization, optical density measurements were performed at 400 nm using a Hewlett Packard 8452A Diode Array spectrophotometer. The sample temperature was held at 30 °C with an external water bath. For the initial optical density measurements, the lipid/cholesterol mixtures were hydrated with 2850 μl water. Following these measurements, 150 μl of ~10% Triton X-100 was added to each sample. Optical density values were then taken ~24 h after the detergent was added (no adjustment was made for dilution by the Triton X-100 solution). With the detergent solution added, the final lipid concentration was 500 nmol/ml.

2.2.3. Fluorescence spectroscopy

The fluorescence samples were prepared as above, but instead contained 3 ml of solution with a lipid concentration of 1 μmol/ml. All samples were placed in a quartz cuvette with a magnetic stirring bar. To prevent oxygen quenching, the solutions were bubbled with nitrogen for at least 15 min. The fluorescence measurements were carried out using an ISS K2 Multi-Frequency Cross-Correlation Phase and Modulation Fluorometer with a Xenon Arc Lamp operating at 15 amps. Excitation was carried out at 360 nm and the emission was measured at 430 nm. A Hoya U-360 bandpass filter was placed in the excitation path and a >400 nm cutoff filter was placed in the emission path to minimize light scattering unrelated to fluorescence emissions. The samples were allowed to cool to room temperature after the initial preparation at 60 °C. Measurements were performed at 30 °C and 55 °C using a circulating water bath. At least two sets of fifteen integrations were obtained for the fluorescence intensity experiments, with the averages of these