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The interdigitated gel phase in mixtures of cationic and zwitterionic phospholipids

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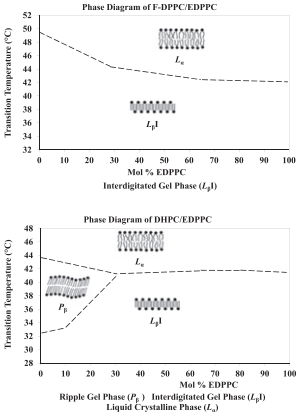
HIGHLIGHTS

- EDPPC, DHPC, and F-DPPC spontaneously form the interdigitated gel phase.
- Mixtures of F-DPPC/EDPPC are interdigitated below the main transition.
- EDPPC stabilizes the interdigitated gel phase of DHPC.
- Lower miscibility when interdigitated and non-interdigitated gel phases are present.
- Cationic and zwitterionic lipid mixtures can be compatible with interdigitation.

GRAPHICAL ABSTRACT



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ABSTRACT

To examine the phase behavior of mixtures of zwitterionic and cationic lipids we used three derivatives of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). All three lipids are uniquely capable of spontaneously forming the interdigitated gel phase (L_βI) under typical hydration conditions. The P-O-ethyl derivative, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (EDPPC), was chosen as the cationic lipid. For the zwitterionic lipids, we use the ether-linked 1,2-di-O-hexadecyl-*sn*-glycero-3-phosphocholine, (DHPC) and the fluorine substituted 1-palmitoyl-2-(16-fluoropalmitoyl)-*sn*-glycero-3-phosphocholine (F-DPPC). Differential scanning calorimetry (DSC) and fluorescence spectroscopy were used to analyze the lipid mixtures. The F-DPPC/EDPPC mixtures are interdigitated at all lipid ratios below the main transition temperature (T_m). In addition, EDPPC stabilizes the interdigitated gel phase of DHPC until the ripple gel phase ($P_{\beta'}$) is eliminated and only the L_βI to liquid crystalline phase (L_α) main transition remains. These results demonstrate that mixtures of cationic and zwitterionic lipids can be compatible with the interdigitated phase.

Abbreviations: DSC, differential scanning calorimetry; DHPC, 1,2-di-O-hexadecyl-*sn*-glycero-3-phosphocholine; DHPE, 1,2-di-O-hexadecyl-*sn*-glycero-3-phosphoethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; EDPPC, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine; Ethyl-PC, ethylphosphocholine; F-DPPC, 1-palmitoyl-2-(16-fluoropalmitoyl)-*sn*-glycero-3-phosphocholine; L_α, liquid crystalline phase; L_βI, interdigitated gel phase; $P_{\beta'}$, ripple gel phase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; T_m, main transition temperature; T_p, pretransition temperature.

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1. Introduction

In the interdigitated gel phase (L_{β}I), the lipid hydrocarbon chains interpenetrate to maximize van der Waals interactions and to reduce head group crowding [1,2]. However, there is also increased unfavorable exposure of the hydrocarbon chains to the surrounding aqueous solution [1–3]. The interdigitated phase has unique properties that are useful for biophysical applications. For instance, the interdigitation-fusion procedure uses ethanol [4,5] or pressure [6] to create high volume vesicles or multi-compartment vesosomes [7]. When interdigitated and non-interdigitated domains coexist the instability and uneven packing at the interface can affect permeability, membrane shape, and fusogenicity [8–10]. Therefore, it is important to test the properties of lipid mixtures, especially those capable of interdigitation.

The naturally occurring lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) does not spontaneously interdigitate, but can be induced to interdigitate by the addition of chemical inducers such as alcohols or by the application of hydrostatic pressure [1,2,11,12].

The ether-linked analogue of DPPC, 1,2-di-O-hexadecyl-*sn*-glycero-3-phosphocholine, (DHPC) and the terminally fluorine substituted 1-palmitoyl-2-(16-fluoropalmitoyl)-*sn*-glycero-3-phosphocholine (F-DPPC) are unique among zwitterionic lipids as they do not require chemical inducers or pressure to interdigitate. DHPC spontaneously interdigitates but the ester-linked DPPC does not because DHPC has relatively higher head group repulsion [13,14], can pack more tightly [15–18], and has weaker attractive interactions between lipids [19,20]. DHPC exists in the L_{β}I phase below the pretransition temperature (T_p) at ~32 °C [19,21,22]. Above the pretransition, DHPC forms the non-interdigitated rippled gel ($P_{\beta'}$) phase. At the main transition (T_m) at ~43 °C, DHPC enters the non-interdigitated L_{α} phase. F-DPPC remains fully interdigitated below the main transition and has no pretransition. The primary reason F-DPPC is fully interdigitated below the main transition (~49 °C) is because the highly polar C-F bond at the end of the *sn*-2 acyl chain is less hydrophobic than DPPC [23,24]. This stabilizes interdigitation since the terminal ends of the acyl chains are exposed within the L_{β}I phase.

The cationic lipid ethyl-phosphocholine (ethyl-PC) derived from DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (EDPPC), shares the ability to spontaneously interdigitate [25,26]. EDPPC interdigitates because of charge repulsion and the high steric bulk of the head group [3,25,26]. Additionally, the ethyl group may reduce the unfavorable contact of hydrophobic acyl chains with the aqueous phase due to a change in the polarity of the lipid molecule [3,26]. The addition of the ethyl group to the phosphate oxygen may also reduce the attractive force of intermolecular hydrogen bonding [27,28]. Compared to the equivalent PC lipids, ethyl-PCs have greater gel phase stability, higher main transition (T_m) temperatures, and greater main transition enthalpy [3]. EDPPC forms a tightly packed L_{β}I phase below its main transition to the liquid crystalline phase (L_{α}) phase at ~42 °C [3,25].

In this study, we examine the miscibility and phase behavior of the cationic EDPPC with two zwitterionic lipids: F-DPPC and DHPC. Cationic lipids have attracted a great amount of interest for their potential as DNA transfecting lipoplexes [10,29,30], transfection reagents for RNA [31,32], co-adjuvants for vaccines [33,34] and as facilitators for drug delivery [35]. Ethyl-PC lipids are of particular interest due to their relatively low toxicity, which is likely a result of their similarity to cellular lipids and because they can be metabolized [28,36]. Intriguingly, DNA can enter into a superlattice between sheets of ethyl-PC lipid without disrupting the interdigitated phase [37,38]. The transfection efficiency of cationic lipoplexes is strongly dependent on the constituent lipids and the resulting phase behavior [10,29,30]. Therefore, it is important to understand the properties of lipid mixtures containing cationic lipids.

2. Materials and methods

All samples were hydrated using purified and double-deionized water from a Milli-Q filtration system. The lipids 1,2-di-O-hexadecyl-*sn*-glycero-3-phosphocholine, (DHPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), the fluorinated 1-palmitoyl-2-(16-fluoropalmitoyl)-*sn*-glycero-3-phosphocholine (F-DPPC), and the cationic 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (EDPPC) were purchased from Avanti Polar Lipids (Alabaster, AL). The fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), was obtained from Molecular Probes (Eugene, OR). Methanol (≥99.9%) was purchased from Sigma–Aldrich (St. Louis, MO). All purchased chemicals were used without further purification.

The DSC and fluorescence samples were prepared from stock solutions of lipid dissolved in methanol. All of the samples were evaporated to dryness followed by high vacuum for at least 10 h. Each sample was then hydrated, incubated at 60 °C, and vortexed periodically for 1 h. Afterwards, all samples were incubated at 25 °C for ~ 24 h after the initial preparation at 60 °C.

The calorimetry samples contained 2 mg lipid in 100 μl of water. The thermograms were obtained on a Calorimetry Sciences Corporation multi-cell DSC-HT Model 4100 DSC. Heating and cooling scans were recorded from 25 °C to 60 °C at a rate of 10 °C/h. The standard deviation of the phase transitions was ±0.1 °C.

The fluorescence samples had a lipid concentration of 1 μmol/ml with a DPH to lipid ratio of 1:500. To prevent oxygen quenching, the solutions were bubbled with nitrogen for 15 min before use. The fluorescence experiments were conducted using an ISS K2 Multi-Frequency Cross-Correlation Phase and Modulation Fluorometer with a Xenon Arc Lamp operating at 15 A. Excitation was carried out at 360 nm and the emission was measured at 430 nm. For the intensity experiments, a Hoya U-360 bandpass excitation filter and a >400 nm cutoff emission filter were used to minimize light scattering unrelated to fluorescence emissions. The filters were removed when measuring fluorescence polarization. A programmable circulating water bath maintained a scan rate of 10 °C/h from 25 °C to 60 °C.

3. Results

3.1. Differential scanning calorimetry

The DSC thermograms of the heating and cooling transitions of F-DPPC/EDPPC are shown Fig. 1A. The main transition temperature (T_m) of EDPPC is 42 °C and the T_m of F-DPPC is 49 °C. Within the temperature range studied no other thermal transitions are observed. Both pure EDPPC and F-DPPC have a large main transition hysteresis of around 4 °C between heating and cooling. This large hysteresis is preserved at all lipid ratios of the F-DPPC/EDPPC mixtures. The T_m decreases steadily up to about 50% EDPPC and approaches the T_m of pure EDPPC (Fig. 2A). Below 50% EDPPC, the transition peaks of the mixtures are broader and some thermograms have shoulder peaks. Above 50% EDPPC, there is little change in the T_m and there is only one sharp heating and cooling peak.

The thermograms of the DHPC/EDPPC system are shown in Fig. 1B. The main transition of DHPC occurs at 43 °C. Unlike the main transition of F-DPPC and EDPPC, the main transition of DHPC is highly reversible with a hysteresis of only ~0.5 °C. Up to 50% EDPPC, shoulder peaks are sometimes present in the heating or cooling thermograms. Above 50% EDPPC there is only a single sharp peak and there is an increase in the main transition hysteresis. The T_m decreases slightly in the mixtures, indicating that the gel phase is destabilized slightly relative to the pure lipids. DHPC also has a low enthalpy pretransition (T_p) that occurs at 32 °C. Fig. 3 shows the DSC thermograms of DHPC/EDPPC up to 30% EDPPC. The pretransition temperature increases with greater fractions of EDPPC (Figs. 2B and 3). Above 30 % EDPPC, the low enthalpy