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Effects of *cis*- and *trans*-unsaturated lipids on an interdigitated membrane

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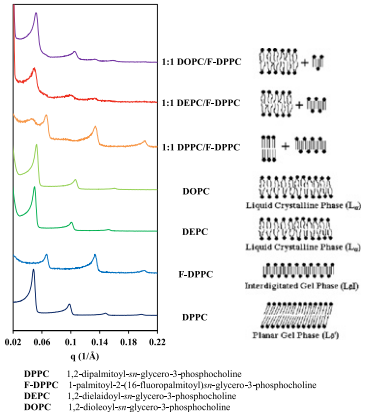
HIGHLIGHTS

- Unsaturated lipids progressively disrupt intermolecular packing.
- Interdigitated and non-interdigitated gel phase coexistence is present.
- The *cis* isomer inhibits interdigitation more effectively than the *trans* isomer.
- Interdigitated phase of F-DPPC resilient in the presence of unsaturated lipid.
- *Trans* lipid properties are intermediate between saturated and *cis*-unsaturated.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of adding *cis*- and *trans*-unsaturated lipid to a fully interdigitated membrane were examined using differential scanning calorimetry (DSC) and X-ray diffraction. A monofluorinated analog of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was used as the interdigitated lipid. The single fluorine atom on the end of the *sn*-2 chain allows 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine (F-DPPC) to spontaneously form the interdigitated gel phase (L_{β}I) below the main transition temperature (T_m). The *cis* 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and equivalent *trans* lipid 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC) are strongly disfavored to form the L_{β}I phase. Wide-angle X-ray scattering (WAXS) data demonstrate that the unsaturated lipids progressively disrupt the intermolecular packing at higher concentrations. Small-angle X-ray scattering (SAXS) data show that as the ratio of unsaturated lipid increases, the amount of interdigitated lipid decreases. The *cis* isomer is more disruptive and inhibits interdigitation more effectively than the *trans* isomer.

Abbreviations: DEPC, 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine; DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DHPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine; DLPE, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; EDOPC, 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine; EDPPC, 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine; F-DPPC, 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine; PVP, poly(vinylpyrrolidone); *D*, lamellar repeat period; DSC, differential scanning calorimetry; SAXS, small-angle x-ray scattering; WAXS, wide-angle x-ray scattering; H_{II}, inverted hexagonal phase; L_{α}, liquid crystalline phase; L_{β}I, interdigitated gel phase; T_m, main transition temperature.

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1. Introduction

Eukaryotes selectively synthesize *cis* fatty acids (CFAs), but *trans* fats acids (TFAs) readily incorporate into phospholipids when provided from exogenous sources [1–4]. Human dietary sources typically come from meat and dairy products from ruminant animals and various hydrogenated or deodorized fats, oils, margarines, and shortenings [5–7]. Consumption of TFAs has been linked to negative health effects [for a review, see [8–11] and references therein]. For instance, TFAs alter the plasma lipoprotein profile by increasing plasma low-density lipoprotein (LDL) cholesterol and decreasing high-density lipoprotein (HDL) cholesterol [12].

However, the mechanism for deleterious health effects is largely unknown [1,12]. It has been hypothesized TFAs may affect membrane structure in a way that alters the activity of proteins [1,3,12–15]. Changing the composition of the lipid membrane can affect protein activity by changing the hydrophobic region [16], domain formation [12], membrane fluidity [1], or acyl chain packing [1,3,4]. Additionally, cholesterol has a higher affinity for TFA- than CFA-phospholipids, which may have an influence on cholesterol-rich membrane "rafts" [3,4].

Using an interdigitated system as a model membrane is an efficient way to gauge the influence of unsaturated lipids on surrounding lipids because it can be easily distinguished from a non-interdigitated membrane. The interdigitated gel phase is characterized by the interpenetration of tightly packed acyl chains, resulting in drastically reduced membrane thickness [17,18]. This structure reduces head group crowding and increases van der Waals forces [19,20]. F-DPPC was selected because it requires no chemical inducers for interdigitation and because it is structurally the same as its parent lipid DPPC except for a single fluorine atom on the end of the *sn*-2 chain (Fig. 1). The spontaneous interdigitation of F-DPPC under typical hydrated conditions can be attributed to the highly polar C–F bond that minimizes the unfavorable interaction of the hydrocarbon chains with the aqueous solvent [17,18,21].

It is also of interest to understand the behavior of fluorine-labeled lipids as they are used as biophysical probes. Fluorine-labeled lipids have been used for ^{19}F NMR techniques [22,23], including use as a rotational-echo double-resonance probe [24], and as a label for secondary ion mass spectrometry [25]. Furthermore, alcohol- or pressure-induced interdigitation is an intermediate step in the interdigitation-fusion process for making vesicles of high internal volume [26,27] as well as multi-compartment "vesosomes" [28]. Therefore, it is of interest to understand why unsaturated lipids have been reported to be incompatible with this process [26].

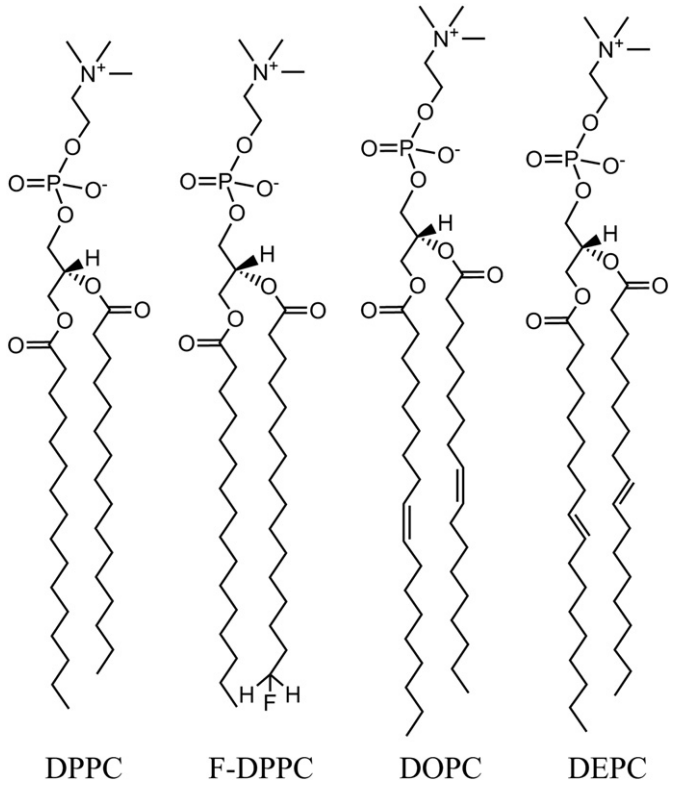

Fig. 1. Chemical structures of DPPC, F-DPPC, DOPC, and DEPC.

2. Materials and methods

The water used in all samples was purified and double-deionized by a Milli-Q filtration system. The lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC), and 1-palmitoyl-2-(16-fluoropalmitoyl)sn-glycero-3-phosphocholine (F-DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Pharmaceutical grade poly(vinylpyrrolidone) (PVP, molecular weight 40,000) was purchased from Sigma–Aldrich (St. Louis, MO).

DSC and X-ray diffraction samples containing different molar ratios of lipids were prepared from combinations of stock solutions of lipid dissolved in methanol. All samples were evaporated to dryness followed by high vacuum overnight to remove any residual solvent. Afterwards, each sample was hydrated and incubated for 1 h at 60 °C with periodic vortexing.

Calorimetry samples contained ~3 mg lipid and were hydrated with 150 μL water. Samples were analyzed with a Calorimetry Sciences Corporation multi-cell DSC-HT Model 4100 DSC at a rate of 10 °C/h. Heating and cooling scans were recorded from 8 °C to 60 °C.

X-ray diffraction samples containing ~6 mg lipid were hydrated in 15 μL PVP (15% w/w) solution such that water is in excess. The use of PVP is known to increase the orders of diffraction detected as it removes water between apposing bilayers thereby compressing the lamellar lattice [17]. These unoriented multilamellar vesicle suspensions were loaded into 1.0 mm quartz capillary tubes. Small- and wide-angle X-ray scattering experiments were performed with a Rigaku Smartlab diffractometer at room temperature (~22 °C). Small-angle data were obtained at a rate of 0.1°/min and wide-angle data were recorded at 1.0°/min. Incident and receiving slits were 0.5 mm for the SAXS measurements and 1.0 mm for the WAXS measurements.

3. Results

3.1. Differential scanning calorimetry

The lamellar crystal phase to liquid crystalline phase transition temperature is −18 °C for DOPC and 12 °C for DEPC. The main phase transition temperature (T_m) of F-DPPC from the interdigitated gel phase to liquid crystal phase occurs at 49 °C. Unlike DOPC and DEPC, there is a significant hysteresis (~4.5 °C) in the main transition temperature of F-DPPC. In the DOPC/F-DPPC system, increasing the portion of DOPC steadily decreases the transition temperature (Fig. 2A). The transition progressively broadens as the DOPC content increases. At high (>75 mol% DOPC) concentrations, no transition is seen within the temperature range studied.

In DEPC/F-DPPC, up to 25 mol% DEPC, the transition broadens and decreases by approximately 5 °C (Fig. 2B). Two separate peaks are visible at equimolar DEPC/F-DPPC. The lower temperature transition has a lower thermal hysteresis and multiple components. Above 50 mol% DEPC, the higher temperature transition disappears and only the broad lower temperature transition remains.

3.2. X-ray diffraction

The small-angle scattering (SAXS) pattern of F-DPPC has single lamellar repeat spacing (D) of ~47 Å (Fig. 3). This is consistent with

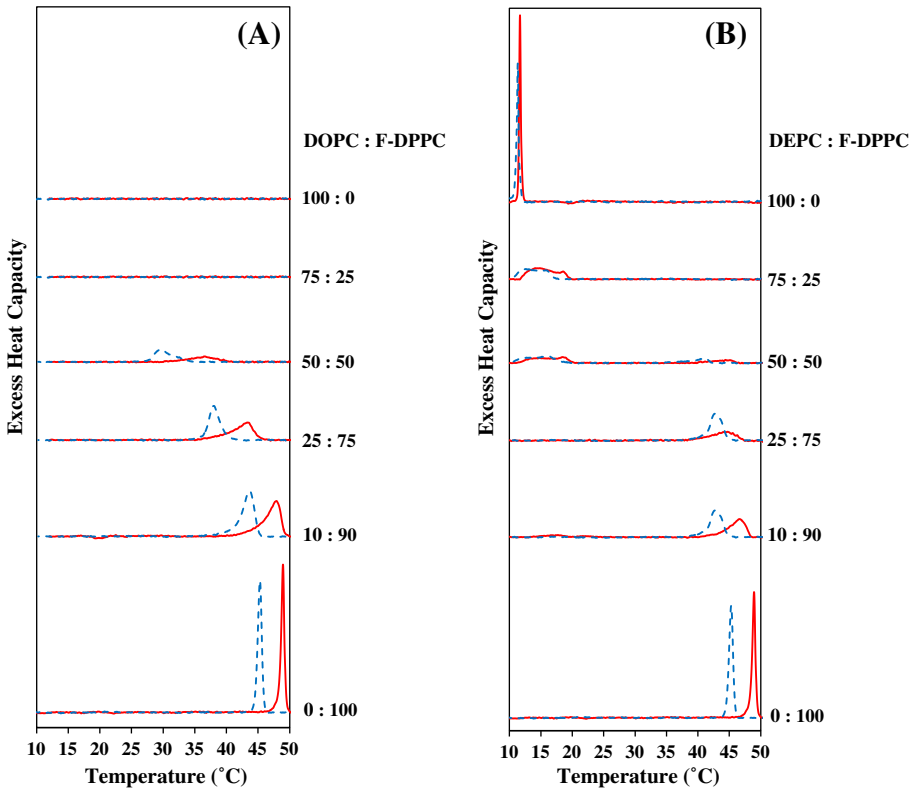

Fig. 2. Representative DSC data of (A) DOPC/F-DPPC and (B) DEPC/F-DPPC. Solid red lines show heating scans and dashed blue lines indicate cooling scans. The cooling scans have been inverted, and the baselines of the thermograms are offset vertically.

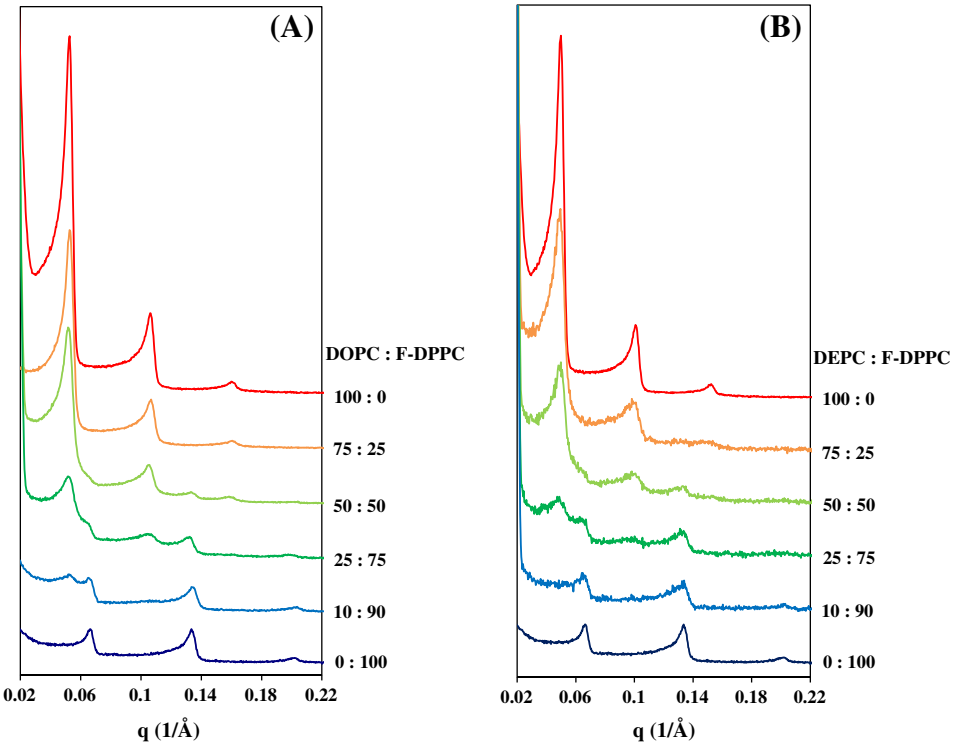

Fig. 3. Small-angle X-ray diffraction pattern of: (A) DOPC/F-DPPC and (B) DEPC/F-DPPC.

the reduced membrane thickness of the interdigitated gel phase [17]. In contrast, the *D* spacings are ~60 Å for DOPC and ~63 Å for DEPC. This is indicative of the non-interdigitated liquid crystalline phase. In DEPC/F-DPPC, there is only one SAXS pattern at 10 mol% DEPC (Fig. 3B). In contrast, a second peak emerges at 10 mol% DOPC with a greater lattice spacing (Fig. 3A). There are two *D* patterns in the 25 mol% DEPC and 25 mol% DOPC samples. The fraction of interdigitated lipid decreases as the amount of unsaturated lipid increases until only the larger *D* spacing remains. Fig. 4 shows a comparison of the unsaturated lipids with DPPC, F-DPPC, and DPPC/F-DPPC. DPPC has a *D* spacing of ~65 Å and two lamellar repeat patterns are present in the equimolar mixture of DPPC/F-DPPC. The ratio of interdigitated lipid with the *cis*- and *trans*-unsaturated isomers is significantly lower compared to saturated DPPC.

A sharp and symmetrical wide-angle scattering (WAXS) peak is present for F-DPPC (Fig. 5). This is representative of the tight hydrocarbon packing in the L_{β}I phase [17,29,30]. The WAXS patterns for DOPC and DEPC are broad, indicating the looser packing and greater spacing between lipids of the liquid crystalline phase. In the lipid mixtures, the broad wide-angle scattering of the liquid crystalline phase overlaps with the sharper gel phase patterns. The portion of the sharp component decreases as the concentration of DOPC and DEPC lipid increases.

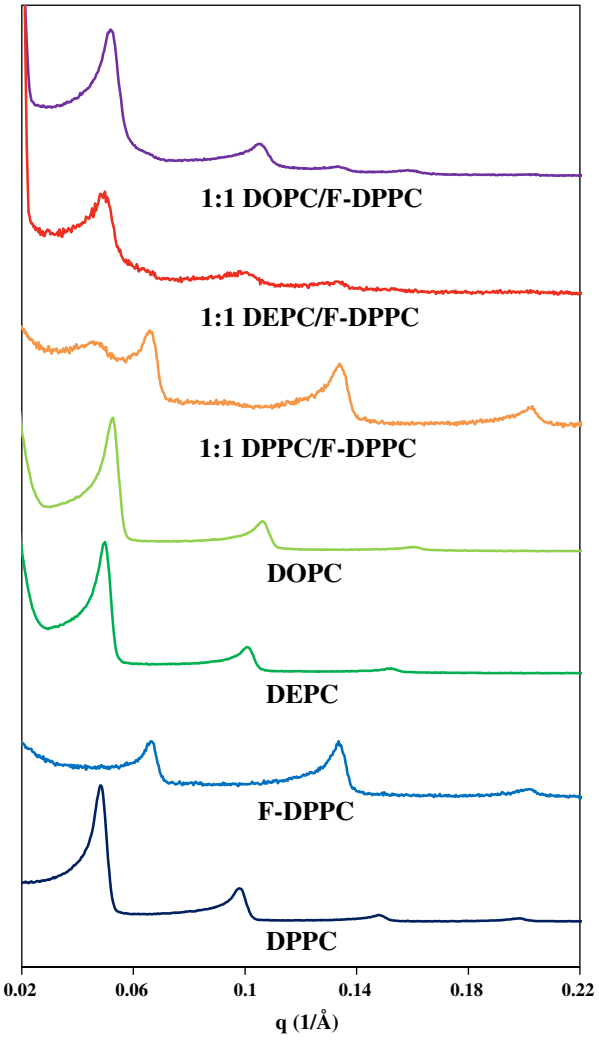

Fig. 4. Small-angle X-ray diffraction pattern of DPPC, F-DPPC, DEPC, DOPC, 1:1 DPPC/F-DPPC, 1:1 DEPC/F-DPPC, and 1:1 DOPC/F-DPPC.

4. Discussion

The main differences of *cis*- and *trans*-lipids occur in the hydrophobic region, while the aqueous interface is similar [31,32]. The *trans* chains can fully extend but the *cis* chains adopt a kinked or bent confirmation whose geometry is more disruptive [3,15,33]. The area/molecule for *cis*-PCs is larger than *trans*-PCs [1]. Both *cis* and *trans* enhance the conformational disorder in the liquid crystalline and gel phases compared to the equivalent saturated lipid [34]. *Trans*-unsaturated lipids have more ordered acyl chain packing than *cis* unsaturation [1–4,32] and some measures of fluidity are greater in *cis* membranes over *trans* membranes [1,3].

As a consequence, the physical properties of membranes made from TFA and CFA lipids are different. A *cis* bond lowers the melting transition temperature to a greater degree than *trans* unsaturation since the looser packing and lower van der Waals interaction destabilize the gel phase [1,15,35]. Additionally, the critical pressure of the liquid-crystalline to gel phase transition is higher in DOPC compared to DEPC, most likely due to difficulty of packing the kinked *cis* double bonds into an organized structure [36]. In the phosphatidylethanolamine (PE) lipids DOPE and DEPE, there is an additional high temperature transition from the liquid crystalline L_{α} phase to the inverted hexagonal phase (H_{II}). The L_{α} to H_{II} transition occurs at a lower temperature for the *cis* versus the *trans* [37]. *Cis*-unsaturated lipids have also been reported to be more permeable than *trans*-unsaturated membranes [1]. Furthermore, *cis*/*trans* isomerism may affect vesicle morphology, curvature, and domain formation [38].

Previous studies have shown that interdigitated/non-interdigitated coexistence can occur in a variety of lipid mixtures. For example, DOPC inhibits the alcohol-induced interdigitation of DPPC [39,40]. Additionally, lateral phase separation occurs between ethanol-induced interdigitated DPPC and non-interdigitated DLPE [41]. Cholesterol inhibits interdigitation in F-DPPC [42], 1,2-dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (EDPPC) [43], and DPPC/ethanol [44], which results in a phase separated system at intermediate cholesterol concentrations. The presence of a lipid capable of interdigitation does not always mean there will be a large degree of coexistence, however. Above 50 mol% DHPC in DPPC/DHPC, the membrane switches from non-interdigitated to a fully interdigitated gel phase with little or no phase coexistence [45]. A similar effect is seen with DPPC/EDPPC where there is little interdigitated/non-interdigitated coexistence [43].

Coexisting interdigitated and non-interdigitated phases are considered to be energetically unfavorable due to the large difference in membrane thickness and lipid organization [46,47]. The structure defects at the boundaries between domains result in increased permeability [41,48,49]. Furthermore, permeability is reported to be greater in coexisting interdigitated gel/non-interdigitated gel phases than with interdigitated gel/liquid crystalline domains [41]. This was explained as a difference in the membrane thicknesses and stability of the boundary regions [41].

Our DSC and X-ray diffraction results show that DOPC and DEPC inhibit interdigitation and promote phase segregation. A large hysteresis between the heating and cooling transitions is indicative of the fully interdigitated phase in pure F-DPPC [21,50]. Consequently, the disappearance of the high temperature, high hysteresis phase transition peak in the DSC scans at high concentrations of unsaturated lipid is consistent with the elimination of interdigitated lipid (Fig. 2).

The presence of two SAXS lamellar repeat (*D*) spacings provides strong evidence for phase coexistence, although the *D* patterns have to be different enough so that individual peaks can be detected [42,45,51]. By these criteria, our X-ray data are sufficient to distinguish between interdigitated and non-interdigitated domains (Figs. 3 and 4). The large change in the *D* spacing between interdigitated (~47 Å) and non-interdigitated phases (~60-65 Å) reveals the difference in membrane thickness [45]. A gradual decrease in the *D* distance may be caused by a change in the lipid tilt or in the thickness of the water

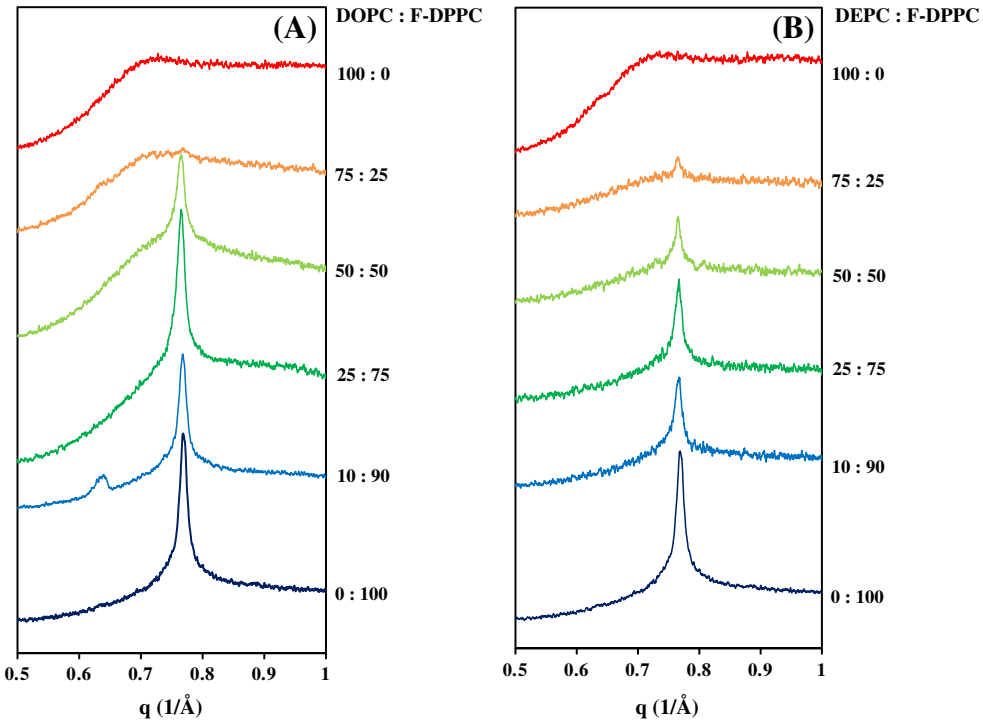

Fig. 5. Wide-angle X-ray diffraction pattern of: (A) DOPC/F-DPPC and (B) DEPC/F-DPPC. Data smoothed using a moving average trend line.

layer. However, the magnitude and suddenness of the change in the *D* spacing is indicative of the presence of interdigitated gel phase, especially since the difference in thickness is consistent with atomic force microscopy measurements [18]. These results also give a qualitative measure of how the ratio of interdigitated lipid decreases with greater unsaturated lipid content. A similar SAXS pattern was discovered in F-DPPC/cholesterol [42].

The unsaturated lipids DOPC and DEPC have the same PC head group and similar acyl chain length to DPPC. The main consequence of unsaturation is the disruption of acyl chain packing, as shown in the WAXS results. WAXS experiments measure the chain–chain correlation spacing and allow for the distinction between gel and liquid crystalline phases [51,52]. Although the liquid crystalline pattern is broad and overlaps with the gel phase peak, it is clear that increasing the unsaturated lipid content broadens the WAXS pattern at the expense of the sharp component (Fig. 5). Similar to F-DPPC/cholesterol, the broadening of the wide-angle peak is consistent with the elimination of interdigitated lipid [42]. The more disordered packing and greater spacing between lipids with higher amounts of unsaturated lipids disfavors interdigitation [20].

When considering the interdigitated gel phase, lipids can be placed into three categories: (1) spontaneous interdigitation, (2) chemical- or pressure-induced interdigitation, and (3) lipids that do not interdigitate even under favorable conditions. When F-DPPC is mixed with the spontaneously interdigitating ether-linked DHPC miscibility is high [53]. In the DPPC/F-DPPC mixture, there are F-DPPC-rich interdigitated domains and DPPC-rich non-interdigitated domains [21]. As DPPC can be induced to interdigitate, it is understandable that it is relatively more compatible with interdigitated F-DPPC compared to unsaturated lipids [19,20].

Membranes made from only unsaturated lipids are highly unlikely to interdigitate. For instance, no ethanol-induced interdigitation occurs in DEPC [54] or DOPC [55]. Similarly, while DPPC can form a pressure-induced interdigitated phase, similar *cis*- and *trans*-unsaturated lipids do not [26,34,36]. Also, the saturated cationic lipid EDPPC spontaneously interdigitates while the *cis*-unsaturated EDOPC does not [56,57]. Consequently, it is unsurprising that DOPC and DEPC have low miscibility with F-DPPC, given the poor miscibility of unsaturated lipids with DPPC [52,58,59]. From our results, it can also be seen that unsaturated lipids are largely incompatible with the interdigitated phase. Significantly more of the membrane in equimolar DPPC/F-DPPC remains interdigitated compared to both DOPC/F-DPPC and DEPC/F-DPPC (Fig. 4). However, more DEPC can be incorporated into interdigitated F-DPPC than DOPC (Fig. 3), indicating that the *trans* isomer is slightly more compatible with interdigitation.

5. Conclusions

The phase diagrams for many lipids have been shown to contain lateral phase segregation [60,61]. In particular, the equimolar mixture of DEPC/DPPC has been used as a model for gel/fluid coexistence [58,59]. Proteins have been shown to segregate based on a preference for either the gel or fluid phases [59,62]. Our results show that domain formation is dependent on saturated, *cis*-unsaturated, and *trans*-unsaturated acyl chains. This supports the hypothesis that the introduction of *trans*-unsaturated phospholipids has the potential to modify membrane properties and the activity of membrane-embedded proteins.

The properties of *trans* fall somewhere in between their saturated and *cis*-unsaturated analogues. However, some characteristics of *trans* lipids behave more like the saturated counterparts while other properties are more similar to the *cis* isomer. In the context of phase segregation, we find that the *trans* lipids behave more like their *cis* counterparts. These findings support that nuance is required in the consideration of *trans* lipids.

We have also found that miscibility for F-DPPC is highest for other spontaneously interdigitating lipids, lower for lipids that can be induced to interdigitate, and lowest for lipids that do not interdigitate. We can conclude that the mutual ability of lipids to interdigitate is an important determinant of miscibility. DOPC is slightly more effective at preventing interdigitation than DEPC, which supports that the *cis*-unsaturated DOPC is more disruptive to surrounding lipids. These results also

demonstrate the surprising resilience of interdigitated phase of F-DPPC in the presence of high concentrations of unsaturated lipid. Therefore, phase segregation allows two very different thermodynamic phases to coexist in the same membrane. This is possible because segregation allows unfavorable interactions to be limited mostly to the interfacial regions between domains.

Acknowledgments

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