# Absence of chiral domains in mixtures of dipalmitoylphosphatidylcholine molecules of opposite chirality

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We present calorimetric data for liposome mixtures of *l*-dipalmitoylphosphatidylcholine with perdeuterated hydrocarbon chains (*l*-DPPC-$d_{62}$) and *d*-DPPC at varying molar concentrations and excess water ($\geqslant$30% by wt.) conditions. The data are consistent with a binary system whose components exhibit complete mutual solid solubility. Therefore, the asymmetric ripples observed in racemic dimyristoylphosphatidylcholine bilayers in the $P_{\beta'}$ phase [Katsaras and Raghunathan, Phys. Rev. Lett. **74**, 2022 (1995)] are not the result of pure enantiomer domains. [S1063-651X(97)03402-8]

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## I. INTRODUCTION

Dispersions of saturated phosphatidylcholines in water typically exhibit two reversible thermotropic phase transitions. One is the so-called pretransition ($L_{\beta'} \rightarrow P_{\beta'}$) in which ''rigid'' planar bilayers transform into periodically modulated bilayers (ripples) having a well-defined wavelength [1–3], while the other, termed the main transition ($P_{\beta'} \rightarrow L_{\alpha}$), results in disordering of the hydrocarbon chains [4] and the disappearance of the ''ripples.''

In recent years, the intermediate rippled $P_{\beta'}$ phase has attracted much attention and has been explored experimentally [3,5–7] and theoretically [8,9]. Experiments have shown that this phase is characterized by either symmetric ($\gamma = 90°$) [5] or asymmetric ($\gamma \neq 90°$) [1,3,6,7,10] ripples of wavelength 130–165 Å and in agreement with a recent theoretical study [9]. Using continuum Landau theory, Lubensky and MacKintosh [9] account for all of the observed ripple phases, including the not so commonly reported symmetric ripple phase [5]. However, using x-ray diffraction and aligned dimyristoylphosphatidylcholine (DMPC) multibilayers, Katsaras and Raghunathan [3] found the $P_{\beta'}$ phase of both chiral and racemic bilayers [11] to be characterized by asymmetric ripples ($\gamma = 99° \pm 2°$), in agreement with data from both freeze-fracture [6] and atomic force microscopies [7]. The model proposed by Lubensky and MacKintosh [9] predicts, in achiral systems, three distinct $P_{\beta'}$ phases, all of which are characterized by symmetric ripples. Only chiral bilayers can exhibit, as a function of decreasing temperature, phase transitions from symmetric to asymmetric ripples [9].

The occurrence of asymmetric ripples in racemic bilayers can be accounted for by the Lubensky-MacKintosh model if the *d* and *l* enantiomers within each layer phase separate into chiral domains. It is known that racemate and pure enantiomers form different solid phases [12]. In lipid systems, the in-plane structure of racemic and chiral phosphatidylcholine bilayers [3] and phosphatidylethanolamine monolayers [13] has also been shown to be different. Theoretically, chiral symmetry can be broken in Langmuir monolayers and freely suspended smectic films through a variety of mechanisms, one of which is the formation of chiral domains if the monolayer is composed of a racemic mixture [14]. Such a separation of chiral phases has been observed in an oriented monolayer of tetracyclic alcohol deposited on mica using atomic force microscopy [15]. The aim of this paper is to investigate whether or not spontaneous phase separation into chiral domains occurs in a lyotropic smectic system.

There seems to be calorimetric evidence suggesting that racemic mixtures form ideal mixtures [6]. However, since the main transition temperatures ($T_m$) of both chiral and racemic DMPC and dipalmitoylphosphatidylcholine (DPPC) liposomes occur within 0.1° of each other [16–18], such a conclusion cannot be made unambiguously. On the other hand, *l*-DPPC with perdeuterated hydrocarbon chains (*l*-DPPC-$d_{62}$) has a $T_m$ of 37.75 °C [19] compared to $\approx$41.7 °C for the protonated phospholipid [6,17,18]. Therefore, by studying mixtures of *l*-DPPC-$d_{62}$ and *d*-DPPC using differential scanning calorimetry (DSC) we can demonstrate whether or not DPPC molecules of opposite chirality form ideal mixtures in the $P_{\beta'}$ phase. Nonideal mixing (lateral phase separation) can easily be detected using DSC by the presence of transition peaks at the $T_m$'s of the two species.

## II. EXPERIMENT

Hydrocarbon chain perdeuterated *l*-dipalmitoylphosphatidylcholine (*l*-DPPC-$d_{62}$) and protonated *d*-DPPC were purchased from Avanti Polar Lipids (Alabaster, AL) and Sigma Chemical Co. (St. Louis, MO), respectively, and were used without any further purification. Both lipids were found to be of high purity ($\geqslant$98%) as demonstrated by thin-layer chromatography at a loading of 100 $\mu$g in two different solvent systems ($CHCl_3$-$CH_3OH$-$H_2O$, 65:25:4, by volume and *n*-$C_4H_{10}O$–$CH_3COOH$–$H_2O$, 60:30:20, by volume). *l*-DPPC-$d_{62}$ and *d*-DPPC were mixed in methanol to form mixtures of 0–100 mol % *d*-DPPC. Bulk methanol was removed using a rotary evaporator, while the remainder of the methanol was evaporated by placing the samples under a vacuum for a period of approximately 18 h. Differential scanning calorimetry was performed using a Microcal MC-2 (Amherst, MA) on lipid samples suspended in a 10 mM sodium phosphate buffer at a concentration of 1 mg/ml using a

 

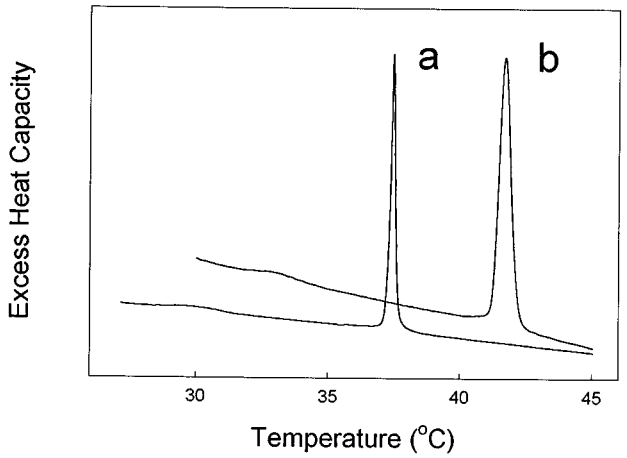


FIG. 1. Differential scanning calorimetry traces for multilamellar suspensions of (a) *l*-DPPC-$d_{62}$ and (b) *d*-DPPC using a scan rate of 13 °C/h.

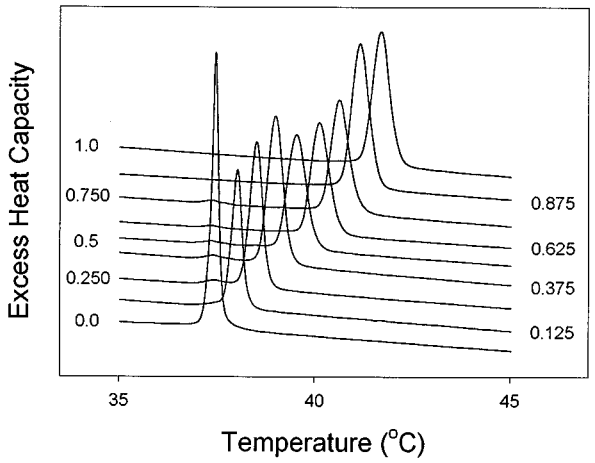


FIG. 2. Main transition ($T_m$) endothermic peaks of mixtures of *l*-DPPC-$d_{62}$ and *d*-DPPC in increments of 0.125 mol fraction of *d*-DPPC. Increasing amounts of *d*-DPPC result in a monotonic increase to $T_m$. The small peak at 37.5 °C, present in some of the scans, is the result of unmixed *l*-DPPC-$d_{62}$ and makes up $\leqslant 1\%$ of the sample.

heating rate of 13 °C/min. Three scans were performed for each lipid mixture.

### III. RESULTS AND DISCUSSION

The calorimetric transition curves of *l*-DPPC-$d_{62}$ and *d*-DPPC multilamellar suspensions are presented in Fig. 1. The main transition temperatures ($T_m$) of *l*-DPPC-$d_{62}$ and *d*-DPPC were 37.45 °C and 41.69 ± 0.05 °C, respectively, and in good agreement with published values [6,19]. In addition, the $P_{\beta'} \rightarrow L_{\alpha}$ transition of *l*-DPPC-$d_{62}$, compared to *d*-DPPC, is sharper (Fig. 1 and Table I) and is indicative of a higher-purity sample.

Figure 2 shows the main transition endothermic peaks for the various mixtures of *l*-DPPC-$d_{62}$ and *d*-DPPC, while the complete scan characteristics are presented in Table I. Besides the main transition peak there is a very weak peak ($\leqslant 1\%$ of sample) arising from unmixed *l*-DPPC-$d_{62}$. This is not the result of chiral phase separation as we do not observe a transition peak in any of the mixtures that can be attributed to pure *d*-DPPC. The main transition of *l*-DPPC-$d_{62}$ liposomes show a systematic increase in $T_m$ with increasing molar proportions of *d*-DPPC. Also, there is a noticeable increase in the full widths at half maximum of the main transition, up to a molar composition of 0.5 (Table I). The above features are characteristic of a system exhibiting complete mutual solid solubility.

Calorimetric studies of racemic phosphorylcholine multibilayers composed of symmetric, saturated acyl chains (e.g., DMPC and DPPC) have shown the excess heat capacity of the subtransition ($L_{c'} \rightarrow L_{\beta'}$) to being either greatly reduced [18], compared to chiral bilayers, or altogether absent. The pretransition temperatures exhibited by racemic samples are also very different from their chiral counterparts [6,16–18]. On the other hand, the main transition temperatures and enthalpies of transition of chiral and racemic bilayers were found to be, for the most part, practically indistinguishable [6,16–18]. This would imply that chiral and racemic bilayers have different structures in the $L_{c'}$ and $L_{\beta'}$ phases, but a similar structure in the $P_{\beta'}$ phase. Support for this can be found in the x-ray study of Katsaras and Raghunathan [3], in which both chiral and racemic aligned DMPC multibilayers had similar $P_{\beta'}$ structures, while their $L_{\beta'}$ phases were structurally different. Unfortunately, although the structure of chiral $L_{c'}$ bilayers has been elucidated recently [20], the struc-

TABLE I. Calorimetric data of mixtures of *l*-DPPC-$d_{62}$ and *d*-DPPC multilamellar suspensions.

| | Pretransition | | Main transition | | |
|---|---|---|---|---|---|
| % *d*-DPPC | $T_p$ (°C) | $\Delta H_p$ (kJ/mol) | $T_m$ (°C) | $\Delta H_m$ (kJ/mol) | FWHM (°C) |
| 0.0 | 29.9 | 4.31 | 37.45 | 35.28 | 0.24 |
| 12.5 | 29.8 | 4.81 | 38.01 | 27.17 | 0.33 |
| 25.0 | 30.3 | 4.60 | 38.50 | 32.69 | 0.39 |
| 37.5 | 30.2 | 5.77 | 38.99 | 42.13 | 0.46 |
| 50.0 | 30.7 | 4.56 | 39.54 | 39.38 | 0.60 |
| 62.5 | 31.2 | 4.56 | 40.12 | 37.54 | 0.56 |
| 75.0 | 31.7 | 4.60 | 40.64 | 36.99 | 0.55 |
| 87.5 | 32.4 | 5.10 | 40.19 | 46.10 | 0.52 |
| 100.0 | 33.4 | 5.81 | 41.69 | 42.93 | 0.51 |

ture of racemic bilayers in the same phase has not.

One assumption that we have made is that isotopic substitution does not alter the interactions between the hydrocarbon chains. Using small-angle neutron scattering, Knoll, Ibel, and Sackmann [21] demonstrated that DMPC-$d_{54}$ with perdeuterated hydrocarbon chains mixed homogeneously with protonated DPPC in both the gel and liquid-crystalline phases and in agreement with calorimetric [22] and spectroscopic [23] data using protonated DMPC and DPPC. Therefore, there seems to be strong evidence that the asymmetric ripple observed by Katsaras and Raghunathan [3] using a racemic mixture of DMPC multibilayers was not the result of the racemate separating into domains of pure *l* and *d* enantiomers. As such, the asymmetry of the ripple cannot be related simply to the chirality of the lipid molecule as predicted by the phenomenological model put forth by Lubensky and MacKintosh [9]. As mentioned previously, chirality has clearly been shown to influence the packing of hydrocarbon chains in phosphatidylethanolamine monolayers [13]. Also, the pitch in thermotropic cholesterics is sensitive to the enantiomorphic composition and is completely absent in racemic mixtures [24]. Consequently, it would be naive on our part to state that chirality has no effect on bilayer structure. However, it seems that any effect of chirality on the structure of the ripple phase in lipid bilayer systems is either subtle or nonexistent.

## IV. CONCLUSION

In this Brief Report we have shown that protonated and deuterated DPPC molecules of opposite chirality exhibit complete mutual solid solubility and do not phase separate into chiral domains. The fact that racemic and chiral phosphatidylcholine bilayers form asymmetric membranes indicates that in such systems, molecular chirality does not seem to be an important factor in determining the presence or absence of ripple asymmetry.

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