Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926090

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To cite this Article Lockwood, Nathan A. , Meli, Maria-Victoria , Surjosantoso, Aditya , Kim, Evelina B. , de Pablo, Juan J. and Abbott, Nicholas L.(2007) 'Characterization of the interactions between synthetic nematic LCs and model cell membranes', Liquid Crystals, 34: 12, 1387 — 1396
To link to this Article: DOI: 10.1080/02678290701682290
URL: http://dx.doi.org/10.1080/02678290701682290

Liquid Crystals, Vol. 34, No. 12, December 2007, 1387–1396

Characterization of the interactions between synthetic nematic LCs and model cell membranes

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(*Received 3 April 2007; accepted in revised form 29 August 2007*)

Differential scanning calorimetry (DSC) was used to characterize interactions of synthetic LCs, 4-pentyl-4′-cyanobiphenyl (5CB) and TL205 (a mixture of cyclohexane-fluorinated biphenyls and fluorinated terphenyls) with simple mimics of cell membranes. The investigation was motivated by reports that living cells can be placed into contact with TL205 without apparent toxicity, whereas contact of cells with 5CB leads to cell death. The tendency was examined for 5CB and TL205 to spontaneously partition into and influence the organization for model cell membranes composed of phospholipids. Upon contact of an aqueous dispersion of DPPC liposomes with neat LC for 4 h, 5CB partitioned into the liposomes at a weight ratio of 5:1 DPPC:5CB, whereas TL205 partitioned at a ratio of 310:1 DPPC:TL205. DSC endotherms indicated that the 5CB spontaneously partitioned into the liposomes was far more perturbing than TL205. DSC endotherms of DPPC bilayers containing the same concentration of either 5CB or TL205 also revealed 5CB to be more perturbing than TL205. The effect of up to 7.8 wt % of TL205 was small, resulting in a shift in the melting transition from 41.4°C to 40.1°C and a minor change in peak width, indicating only minor effects on the organization of the bilayer. These effects are similar to those caused by cholesterol in DPPC bilayers. In contrast, 5CB shifted the DPPC melting transition from 41.4°C to ~36°C and increased the width of the transition peak by a factor of ten, indicating a destabilization of the ordered phase in the bilayer and a disruption of the cooperative nature of the gel-to-LC transition of the phospholipid bilayer. Taken together, the results demonstrate that 5CB and TL205 differ significantly in their interactions with model cell membranes, which suggests one possible origin of their different toxicities toward cells.

1. Introduction

Recently, several studies have reported experiments in which cells were contacted with synthetic LCs [1–3]. These studies sought, in part, to investigate the ordering of LCs in the vicinity of cells as a potential means of reporting the presence of cells or the interactions of cells with their environment. For example, a study by Fang *et al.* investigated the ordering of the nematic LC 4-pentyl-4′-cyanobiphenyl (5CB, figure 1) placed into contact with fixed (i.e. dead) cells attached to surfaces [3]. The authors demonstrated that the ordering of the LC was influenced by the cells in a manner that depended on the type of cell. More recently, LCs have been used in experiments in which the they were placed into contact with living cells. Lockwood *et al.* demonstrated that it was possible to culture human embryonic stem cells on the surface of a film of the nematic LC TL205, a mixture of cyclohexane-fluorinated biphenyls and fluorinated terphenyls (see representative structures shown in figure 1) [2]. The stem cell colonies survived for up to 14 days on the surface of the TL205 without observable signs of toxicity. The authors also demonstrated that the ordering of the LC was influenced by the extracellular matrix of the cells and that reorganization of the extracellular matrix by the stem cells led to ordering transitions in the LCs.

A third study, which is particularly relevant to the experiments reported in this paper, involved measurements of the survival of live cells immersed under a range of LCs that were selected on the basis of their chemical functional groups (ether, ester, cyano, hydrocarbon, fluorocarbon) [1]. This study identified LCs that were toxic or non-toxic to two mammalian cell lines, 3T3 fibroblasts and corneal epithelial cells. In particular, whereas LCs containing fluorocarbon

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Liquid Crystals
ISSN 0267-8292 print/ISSN 1366-5855 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/02678290701682290

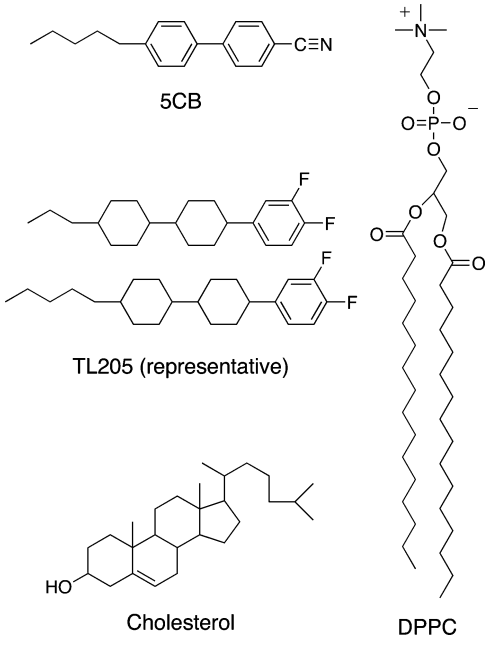

Figure 1. Molecular structures of the molecules used. Liquid crystals include 4-pentyl-4′-cyanobiphenyl (5CB) and TL205, a mixture of cyclohexane-fluorinated biphenyls and fluorinated terphenyls. Representative structures of the components of TL205 are shown. Also shown are cholesterol and the phospholipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPC).

functionalities, among them TL205, were found to be non-toxic, a variety of other LCs, including 5CB, caused cell death within 4 h. The authors also observed that 5CB permeabilized cell membranes, whereas TL205 did not. This observation suggests that one possible mechanism of toxicity, amongst many candidates including apoptotic signalling cascades and disruption of vital enzyme pathways, is disruption of the cell membrane by 5CB and other toxic LCs.

In this paper, we consider the hypothesis that LCs observed to be toxic to mammalian cells (represented here by 5CB) differ from the non-toxic LCs (represented by TL205) in the nature of their interaction with the lipid bilayers of cell membranes. To test this hypothesis, we investigated the interactions of the LCs with multilamellar phospholipid vesicles, a simplified, but commonly employed, model of the cell membrane. We hypothesized that the different toxicities of these two LCs may reflect differences in the tendency of the LC to partition into the membranes and the impact the organization of the lipid bilayer of a cell membrane. We tested this hypothesis by measuring the partitioning of LCs from bulk LC into an aqueous dispersion of multilamellar phospholipid vesicles, and by using differential scanning calorimetry to monitor the effects of LCs on the organization of phospholipid bilayers.

The approaches we used to investigate the interactions between synthetic LCs and lipid bilayers are inspired by past studies of the influence of cholesterol, which is also capable of forming a liquid crystalline phase [4–6], and other small molecules on the organization and physical properties of lipid bilayers [7–9]. One method widely used to probe the interactions of small molecules with phospholipid membranes is differential scanning calorimetry (DSC) [10–12]. DSC studies have revealed the effects of cholesterol on lipid bilayers to include broadening and elimination of the gel to liquid crystalline phase transition of the lipid bilayer, elimination of the gel to rippled gel pretransition, an increase in the orientational order of the acyl chains of the lipid in the liquid crystalline phase, a corresponding decrease in the order in the gel phase and a decrease in the tilt of the acyl chains of the lipid in the gel phase [10–13]. The study reported here also builds from past studies by Kühnau *et al.*, who used DSC in concert with Langmuir-Blodgett and X-ray scattering measurements to investigate the influence of LCs on the organization of lipid monolayers and bilayers [14, 15]. They observed that increased amounts of 5CB incorporated into DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) bilayers leads to a change in the ordering of the tails of the phospholipid. They did not, however, investigate TL205 in this regard. Finally, we note that a number of past studies have demonstrated that phospholipid monolayers on solid [16–19] or fluid [20, 21] substrates can orient thermotropic LC phases. The mechanism of orientation has been suggested to involve a mixing of the LCs with the acyl tail region of the phospholipids.

2. Materials and methods

2.1. *Materials*

DPPC and cholesterol were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). The LCs TL205 and 5CB were obtained from EMSciences (New York, NY). Sodium phosphate, sodium hydroxide, sodium chloride, chloroform, methanol, and hexanes were obtained from Fisher Scientific (Pittsburgh, PA). Sodium dodecyl sulfate (SDS) was obtained from Sigma-Aldrich (St. Louis, MO) and recrystallized from ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) prior to use. Deionization of a distilled water source was performed with a Milli-Q system (Millipore, Bedford, MA) to yield water with a resistivity of 18.2 MΩ cm.

2.2. *Preparation of liposome suspensions*

A chloroform solution of DPPC or a mixture of DPPC and 5CB or TL205 in chloroform was placed in a glass

tube and dried under a flow of nitrogen to form a thin film on the surface of the tube. Residual solvent was removed under vacuum at 50°C for at least 2 h. The resulting film was hydrated overnight at 50°C (above the lipid melting transition temperature) with an appropriate volume of phosphate buffer (50 mM sodium phosphate, pH 7.4) to yield a final lipid concentration of 4 mM for DSC measurements or 1 mM for UV absorption measurements. The solutions were periodically vortexed during the process of hydration. This procedure produced a suspension of large multilamellar vesicles appropriate for DSC measurements [22].

2.3. *UV measurements*

UV absorption measurements were made at room temperature on a Varian Cary 1E UV–visible spectrophotometer (Varian, Inc., Palo Alto, CA). Samples were placed in quartz cuvettes with a 1 cm path length. Extinction coefficients were measured for 5CB and TL205 by preparing solutions of 5CB or TL205 in either hexanes or ethanol and measuring the UV absorbance at five concentrations. A linear fit of the absorption values (at 280 nm for 5CB, 258 nm for TL205) as a function of concentration yielded extinction coefficients of 98.4 and 62.8 $L\,g^{-1}\,cm^{-1}$ for 5CB and TL205, respectively. The coefficients were comparable when measured in hexanes or ethanol.

2.4. *Solubility of LCs in aqueous solutions of buffering salts*

10 μL of 5CB or TL205 was added to 10 ml phosphate buffer (50 mM sodium phosphate, pH 7.4) and equilibrated at 37°C for up to seven days. The mixtures were vortexed approximately hourly for the first day and twice daily after the first day for seven days. The concentration of 5CB or TL205 in the aqueous phase was determined by measurement of the UV absorbance, as described above.

2.5. *Partitioning of LCs into DPPC liposomes*

A 10 ml suspension of multilamellar DPPC vesicles (1 mM DPPC) in phosphate buffer was mixed with 20 μl of 5CB or TL205 and equilibrated at 37°C for up to seven days. The mixtures were vortexed hourly for the first day and twice daily after the first day. The amount of LC that was taken up by the DPPC liposomes was quantified by UV absorbance. Aliquots of the aqueous suspensions of the multilamellar vesicles were removed from the tube with a syringe, taking care to avoid removal of the neat LC, which was visible as an immiscible phase at the bottom of the tube. The suspension was mixed with SDS surfactant (final SDS concentration ~18 mM) to solubilize the lipids and LC. The addition of SDS produced a transparent solution and eliminated problems arising from the scattering of light from large multilamellar vesicles. UV spectra of the resulting solutions were recorded at room temperature.

2.6. *Differential scanning calorimetry (DSC)*

Calorimetric measurements were made with a MicroCal MCS differential scanning calorimeter (MicroCal, Inc., Northampton, MA) with a scan rate of $30°C\,h^{-1}$ and a filter period of 5 s. Scans ranged from 5 to 80°C and were repeated three times for each sample; the second and third runs were typically identical, indicating reversibility and no detectable thermal hysteresis. The concentration of phospholipid in the aqueous suspensions was between 3 and 4 mM. Reference scans (buffer/buffer) were subtracted from each experimental scan prior to baseline correction and concentration normalization.

Analysis and deconvolution of DSC endotherms were performed with MicroCal's Origin software. The software deconvolutes the data by approximating the melting endotherms as a linear combination of multiple independent two-state transitions. This protocol has been used extensively to characterize endotherm changes induced by cholesterol [12, 23–26].

3. Results and discussion

3.1. *Influence of 5CB and TL205 spontaneously partitioned into phospholipid vesicles on the organization of lipids*

Our first experiments involved the use of DSC to determine the influence of 5CB or TL205 spontaneously partitioned into hydrated lipid bilayers on the organization of the bilayers. In past experiments that involved the contact of cells and LCs [1, 2], such a spontaneous partitioning would be necessary for the LCs to impact the organization of the cell membrane. We performed these experiments by contacting 5CB or TL205 with 4 mM suspensions of DPPC multilamellar vesicles in phosphate buffer, pH 7.4 at 37°C (see below for comments on effect of temperature and for results with fluid lipid bilayers). To promote contact between the vesicles and LC, the mixtures were vortexed hourly for the first day and twice daily thereafter for seven days. Prior to contact with the LCs, the suspensions of DPPC multilamellar vesicles appeared turbid. Aqueous suspensions of DPPC vesicles equilibrated in contact with 5CB became noticeably more turbid over the course of days. The appearance of DPPC suspensions that were

not contacted with LC (controls) and DPPC suspensions equilibrated in contact with TL205 did not change over seven days. As discussed in more detail below, the above procedures were adopted to promote contact between the LCs and lipids and to mimic conditions used in past experiments involving contact of cells with LCs. Although these procedures led to droplets of LCs dispersed in the aqueous phase (see below), such droplets gave rise to contributions to the endotherms obtained by DSC that were easily identified. Finally, we note that we observed no apparent decrease in the volume of either 5CB or TL205 coexisting with the aqueous dispersions of vesicles over time, indicating that the amount of LC partitioning into the lipid or aqueous phase was small compared to the volume of LC contacted with the aqueous dispersion of vesicles.

DSC endotherms obtained as described above prior to contact of the DPPC with LC (no 5CB or TL205) were consistent with previous measurements of the endotherms of multilamellar vesicles of DPPC (figure 2) [11, 27]. The endotherms exhibited two transitions: a lower-enthalpy pretransition at 34.0°C and a higher-enthalpy main transition at 41.4°C. The pretransition arises from conversion of a lamellar gel ($L_{\beta'}$) phase, in which the lipids are tilted with respect to the bilayer normal [28], to a rippled gel phase ($P_{\beta'}$), in which the lipids are nearly normal to the interface [29] and the bilayer possesses a long wavelength undulation [30]. The main transition, commonly called the melting transition

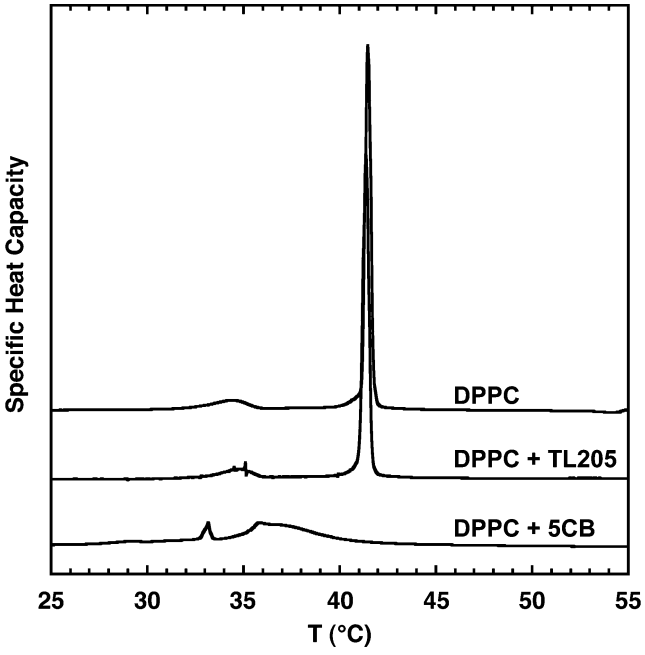

Figure 2. DSC endotherms of DPPC multilamellar vesicle suspensions following seven days of incubation at 37°C in the absence or presence of liquid crystal.

(T_m), arises from the conversion of the $P_{\beta'}$ phase to a lamellar liquid crystalline (L_α) phase. This transition is accompanied by a decrease in the order in the lipid tails (i.e. the development of trans-gauche isomerism in the hydrocarbon tails) and an increase in the mobility of the lipids within the bilayer [31–33].

We observed that the DSC endotherms of DPPC vesicles that had been contacted with TL205 or 5CB differed from the endotherms of DPPC vesicles that had not contacted LC (figure 2). Equilibration of the vesicles with TL205 caused the pretransition temperature of the DPPC to increase from 34.0°C to 34.7°C and the melting temperature to decrease from 41.4°C to 41.3°C. The melting transition showed little change in peak shape or width. In comparison, the transition temperatures of DPPC vesicles incubated in the presence of 5CB changed to a large extent. After equilibration with 5CB, no pretransition was apparent in the DSC endotherms and the melting temperature shifted from 41.4°C to 35.9°C. The peak corresponding to the main transition was also observed to become very broad upon contact with 5CB.

We interpret these DSC results to indicate that 5CB and TL205, which spontaneously partitioned into the lipid bilayers, impacted the organization of DPPC bilayers to substantially different degrees. The large shift in position of the main transition peak caused by the presence of 5CB suggests a destabilization of the more ordered gel phase of the DPPC. In addition, the increase in the breadth of the main transition peak indicates a decrease in the cooperativity of the transition. This suggests that 5CB incorporates into the hydrophobic portion of the bilayer in a manner that creates a variety of microenvironments for the DPPC molecules (i.e. non-uniform mixing of 5CB into the DPPC bilayer) and reduces the cooperative unit of the DPPC transition [13]. These conclusions are consistent with conclusions reached by Kühnau *et al.* in past studies of 5CB incorporated into DPPC bilayers [14]. In contrast to 5CB, the TL205 that spontaneously partitioned into the DPPC vesicles had a small impact on the phase transitions of the DPPC bilayers. The TL205 present in the bilayer caused only minor changes in the shape of the main DPPC transition peak, indicating a small effect of TL205 on the cooperativity of the transition.

We note that a new, sharp transition was observed at 33.5°C in samples incubated in the presence of 5CB (figure 2); we attribute this peak to the nematic-to-isotropic phase transition of bulk 5CB. A peak in the endotherm at 86°C was also observed in samples incubated in TL205 when DSC scans were extended to 90°C (data not shown). The observation of a nematic–

isotropic transition in the DSC endotherm indicates that some LC was suspended in the aqueous phase (due to the vigorous nature of the vortexing during sample preparation). Since the transition temperatures measured in our experiments correspond to the bulk transition temperatures of 5CB and TL205, the suspended LC droplets must be of sufficient size to possess bulk properties; i.e. they must be microscopic rather than nanoscopic droplets. The nematic–isotropic transition temperatures of small volumes and thin films of LCs are typically shifted from the bulk values due to effects of confinement [34].

3.2. *Estimation of the amount of 5CB or TL205 spontaneously partitioned into phospholipid vesicles*

We used UV–visible absorption measurements to estimate the relative amounts of 5CB and TL205 taken up into the aqueous dispersion of DPPC vesicles under the conditions used to obtain the DSC data described above. We observed the uptake of the LCs into the aqueous dispersion of vesicles to be a slow process that was dependent upon the procedure used to contact the bulk LC and aqueous dispersion of vesicles (see appendix). Using the contact procedures described above (vortexing), we determined that the spontaneous uptake of LCs into the aqueous dispersions of DPPC vesicles to be $89 \pm 35\,\mathrm{mg\,l^{-1}}$ for 5CB and $1.5 \pm 0.8\,\mathrm{mg\,l^{-1}}$ for TL205. The concentrations of LC taken up into the dispersions of DPPC correspond to mass ratios of 5.4:1 for DPPC:5CB and 310:1 for DPPC:TL205 (15.7 wt% and 0.3 wt% of 5CB and TL205, respectively). Although some fraction of this LC is present as dispersed droplets (see comments above regarding DSC measurements), the level of uptake of 5CB that we measured is similar to that reported by Kühnau *et al.*, who reported a molar ratio of 2:1 DPPC:5CB (equivalent to a mass ratio of 5:1) to be the upper limit of miscibility of 5CB in DPPC bilayers based on DSC measurements [14].

Our data indicate that the uptake of TL205 into the DPPC vesicles is far less than 5CB (by a factor of approximately 60). This result may reflect the very low solubility of TL205 as compared to 5CB in aqueous buffer ($140 \pm 20\,\mu\mathrm{g\,l^{-1}}$ for 5CB and less than $20\,\mu\mathrm{g\,l^{-1}}$ for TL205), and thus slow transport of TL205 from the bulk to the vesicles within the dispersion. We note that uptake of TL205 likely does not correspond to equilibrium between the bulk TL205 and dispersion of vesicles. The main conclusion to be extracted from our data is that TL205 differs substantially from 5CB in its capacity to be taken up into lipid bilayers and thereby perturb the organization of the lipid within the bilayers.

An important difference between DPPC bilayers and cell membranes (when viewing DPPC as a primitive model for a cell membrane) is that DPPC is in the gel state at 37°C, whereas cell membranes are fluid at physiological temperatures. To determine if the uptake of TL205 or 5CB is substantially dependent on whether the lipids contacted with the LC are above or below their melting temperatures, we contacted aqueous dispersions of either DPPC (T_m=41°C) at 37°C and 50°C or DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) (T_m= 23.7°C) at 37°C with TL205 and 5CB. In these experiments, the extent of uptake of the LC was determined to be only 20% lower at a given time point for experiments performed with DPPC in the gel state as compared to DMPC and DPPC at temperatures above their melting temperatures (see appendix). Importantly, the uptake of 5CB remained substantially greater than TL205.

3.3. *Impact of 5CB and TL205 on the organization of DPPC in vesicles prepared by premixing DPPC with 5CB or TL205*

The results described above suggest that 5CB spontaneously partitioned into DPPC vesicles has a much greater impact than TL205 on the organization of the lipid in the vesicle bilayer. However, the amount of 5CB in the vesicle phase is higher than the amount of TL205 in the vesicle phase. To address the role of this difference in partitioning, we next prepared DPPC vesicles containing identical weight fractions of 5CB and TL205 and used DSC to measure the phase behaviour of DPPC in order to better understand the impact of 5CB and TL205 on the organization of DPPC bilayers. The procedure involved dissolving measured amounts of 5CB or TL205 into chloroform along with the DPPC during preparation of the vesicles in order to establish fixed ratios between the lipids and LCs.

The incorporation of up to 7.8 wt% 5CB into vesicles of DPPC led to significant changes in the DSC endotherms (figure 3 A). Qualitatively, the main DPPC melting peak shifted to lower temperatures and broadened, and the pretransition peak shifted to lower temperatures as the amount of 5CB incorporated in the DPPC bilayers increased (figure 3 A, inset). Over the same range of weight fractions, TL205 caused smaller shifts in the melting temperature of DPPC (figure 3 B). We measured only small increases in both the breadth of the main transition and the pretransition temperature (figure 3 B, inset).

In contrast to samples in which 5CB and TL205 were allowed to spontaneously partition into aqueous dispersions of DPPC vesicles (figure 2), we did not observe peaks in the DSC endotherms corresponding to the nematic–isotropic transition of 5CB or TL205 when

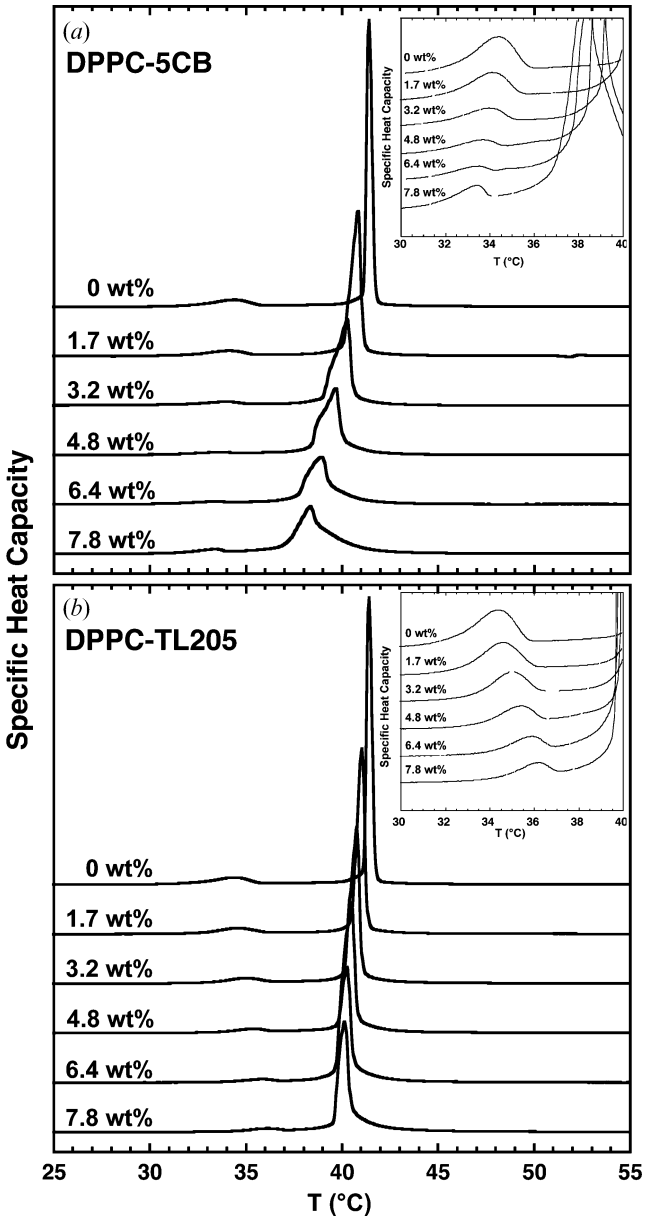

Figure 3. DSC endotherms of DPPC vesicles containing 5CB (A) or TL205 (B). The effects of 5CB or TL205 on the pretransition of DPPC are shown in the insets.

the DPPC vesicles were prepared from chloroform solutions containing 5CB or TL205 (figure 3). This result supports our conclusion regarding the presence of a small amount of bulk LC in the aqueous phase in the partitioning experiments reported above. It also suggests that 5CB and TL205 incorporated into the DPPC vesicles at the time of preparation of the vesicles does not segregate from the lipid into droplets of LC.

It is interesting to note that 5CB and TL205 had opposite effects on the pretransition of the DPPC bilayers (figure 3, insets). 5CB caused a decrease in the pretransition temperature whereas TL205 caused an increase in the pretransition temperature. Physically, this suggests that 5CB stabilizes the rippled gel (P_{β}') phase and TL205 stabilizes the lower-temperature lamellar gel (L_{β}') phase of the DPPC. A significant difference between the P_{β}' and L_{β}' phases is the tilt angle of the lipids relative to the bilayer normal [28–30]. It is therefore likely that the opposite shifts in the pretransition caused by 5CB and TL205 reflect subtle differences in the way in which the LCs influence the tilt angle of the DPPC lipids within the bilayers.

We quantified the effects of 5CB and TL205 on the thermal transitions of DPPC bilayers in order to compare the impact of 5CB and TL205 with that of cholesterol, which has been extensively studied in the past [10–13]. We focused our comparison on the main melting transition of DPPC and examined the influence of 5CB, TL205 and cholesterol on the position of the melting transition, T_m, the width of the melting transition, $\Delta T_{1/2}$, and the enthalpy of the melting transition (figure 4).

Increasing amounts of 5CB, TL205, and cholesterol each caused a decrease in the T_m of DPPC (figure 4 A), which indicates destabilization of the gel phase relative to the liquid crystalline phase of DPPC. However, the effect of 5CB was more pronounced than that of cholesterol, whereas the effect of TL205 was comparable to the effect of cholesterol reported McMullen *et al.* [35]. We note that we also obtained DSC endotherms of DPPC and cholesterol at select concentrations and confirmed that the values we obtained were similar to McMullen and co-workers (data not shown). Our analysis also reveals that 5CB had a larger effect than TL205 or cholesterol on the width of the melting transition of DPPC (figure 4 B), which indicates that the melting transition of the DPPC tails remains a cooperative process in the presence of TL205 or cholesterol, but 5CB disrupts the cooperative nature of the melting process [13]. Finally, we observed little difference in the effect of 5CB, TL205 or cholesterol on the enthalpy of the melting transition of DPPC (figure 4 C). Each species caused a decrease in the enthalpy of the main transition with increased amounts of LC up to 7.8 wt %.

The data presented in figure 4, when combined, suggest that 5CB is more disruptive than TL205 to the organization of the tails in the DPPC bilayer. Notably, the results may also provide insights into the apparent absence of toxicity of TL205 when in contact with cells: The data shown in figure 4 suggest that the effect of TL205 on the organization of DPPC bilayers is indistinguishable from cholesterol, a common component of biological membranes.

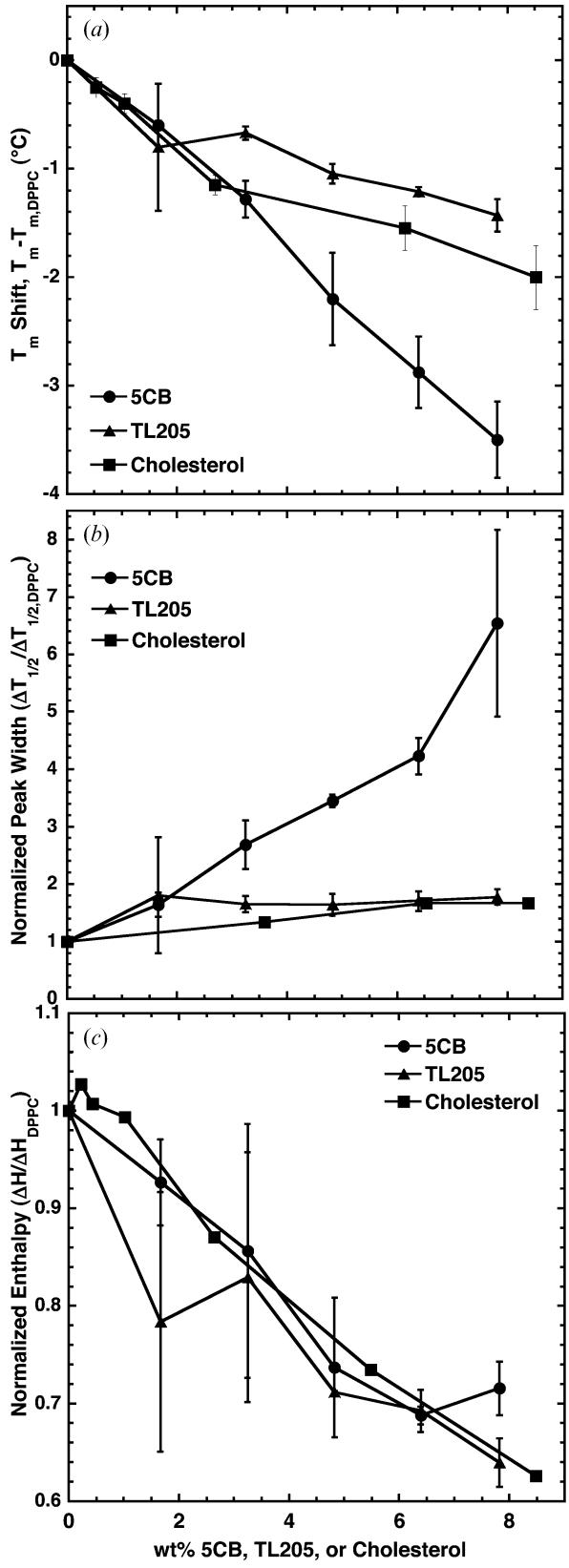

Figure 4. Analysis of DSC endotherm parameters for DPPC mixed with 5CB or TL205. Results for DPPC mixed with cholesterol are shown for comparison (data are taken from McMullen *et al.* [35] and Estep *et al.* [12]). (A) Shift of the main melting transition temperature relative to the melting temperature of pure DPPC (41.4°C). (B) Transition peak width, $\Delta T_{1/2}$, normalized by $\Delta T_{1/2}$ for pure DPPC. (C) Enthalpy of the main transition peak as measured by integration of the area under the peak. The values are normalized to the area under of the main transition measured for pure DPPC.

Finally, we analyzed DSC endotherms by deconvoluting the transition peaks to facilitate further comparison of the influence of 5CB and TL205 on the phase behaviour of DPPC bilayers. Similar analyses of mixtures of cholesterol and DPPC suggest that mixtures of DPPC and cholesterol form a two-phase system [23]: the phase rich in DPPC appears as a sharp peak in the deconvolution of the DSC endotherm, and the phase lean in DPPC appears as a broad peak. The relative contribution of sharp and broad components depends on the amount of cholesterol present in the system; at a sufficiently high concentration of cholesterol, the sharp peak disappears entirely. Deconvolution of endotherms we obtained for mixtures of DPPC and 5CB or TL205 reveal that small amounts of TL205 led to two phases that are manifested as broad and sharp component peaks in the endotherm (figure 5), similar to the behaviour observed for cholesterol [23]. In contrast, higher concentrations of TL205, and all concentrations of 5CB investigated, led to far more complex phase behaviour, as indicated by deconvolution of the main transition peak into up to four component peaks. The nature of these multiple phases cannot be discerned from our experiments, but the phases must correspond to mixtures of DPPC and components of TL205 or 5CB at various fixed weight ratios in each phase. This finding is consistent with results reported by Kühnau *et al.*, who concluded from DSC and X-ray diffraction experiments that mixtures of 5CB and DPPC form mixed phases [14].

The differences in the aqueous solubilities, tendency to partition into lipid vesicles, and impact on the organization of the lipid bilayers that we observed for 5CB and TL205 reflect differences in the chemical structures of these mesogens. For example, the higher aqueous solubility of 5CB as compared to TL205 is due to the polar nitrile functionality and aromatic ring structure of 5CB; these functional groups also likely underlie differences in the ability of TL205 and 5CB to partition into lipid bilayers. Our observation that 5CB disrupted the organization of DPPC bilayers more strongly than did TL205 suggests that 5CB mixes better with the DPPC lipids in the bilayer. The segregation of lipophobic, fluorinated surfactants from hydrocarbon

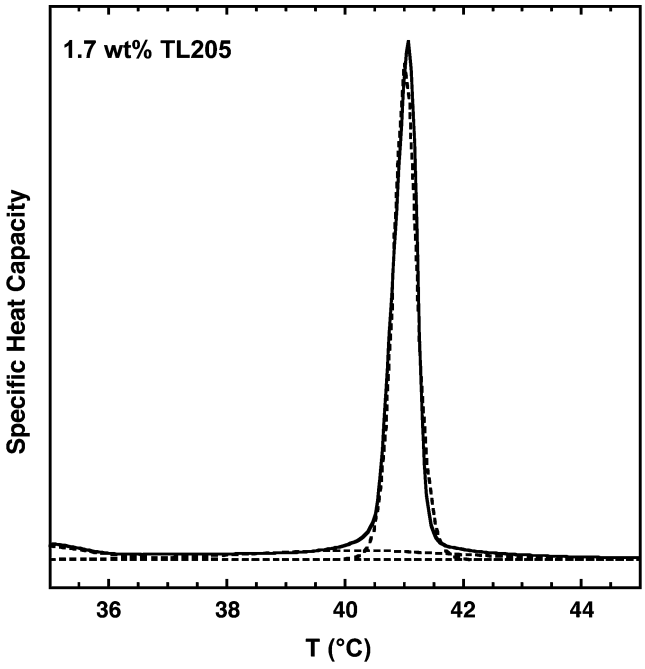

Figure 5. Representative component DSC peaks of the main phase transition of DPPC containing 1.7 wt% TL205. The solid line is the observed DSC endotherm and dotted lines are component curves produced by deconvolution of the observed endotherm.

surfactants has been well established for several fluorocarbon–hydrocarbon mixed bilayer systems (e.g. Yoder *et al.* [36] and references therein). The two cyclohexyl groups of TL205 may impose additional steric and electronic constraints that limit mixing with the aliphatic tails of the DPPC lipid. In contrast, favourable interactions between the polar cyano functionality of 5CB and the polar head group of DPPC may contribute to the improved mixing of 5CB into the lipid bilayer.

Recent molecular dynamics simulations of the interactions of 5CB and an analogue of TL205 (5CF) with DPPC bilayers provide additional insight into how the chemical structures of 5CB and TL205 influence interactions of the mesogens with lipid bilayers [37]. The results of these simulations revealed a driving force for partitioning of 5CB and 5CF into a DPPC bilayer from an aqueous phase, that 5CB and 5CF insert into the hydrophobic portion of the bilayer, and that the presence of 5CB or 5CF tends to increase the ordering of the lipid tails. The data we report here are consistent with these simulations in that UV measurements indicate partitioning of 5CB and TL205 into the DPPC bilayer, and that shifts in the phase behaviour of DPPC by DSC suggest that 5CB and TL205 interact with the hydrophobic region of the lipids. In contrast to the simulations, however, we did not observe evidence of ordering of the lipid tails in the presence of 5CB or TL205. Experimentally, each of the LCs appears to decrease the ordering of the tails, as evidenced by the stabilization of the less-ordered phases of the lipids.

4. Conclusions

In conclusion, the experimental measurements reported in this paper reveal that 5CB and TL205 differ substantially in their interactions with DPPC bilayers in multilamellar vesicles. Our results indicate that 5CB partitions to a greater extent than TL205 into DPPC bilayers and, when present in the bilayer at the same weight fraction, 5CB is more disruptive than TL205 to the organization of the DPPC tails. These results suggest physical mechanisms by which 5CB and TL205 may differ in their interaction with cells. Importantly, our results also suggest that the impact of TL205 on the organization of DPPC bilayers is very similar to cholesterol, which may account for the apparent lack of toxicity of TL205 when placed into contact with living cells.

Acknowledgements

We thank Dr. Carolina Shebor for helpful discussions on experiments and Dr. Darrell McCaslin for his assistance with DSC. DSC data were obtained at the University of Wisconsin-Madison Biophysics Instrumentation Facility, which is supported by the University of Wisconsin-Madison and grants BIR-9512577 (NSF) and S10 RR13790 (NIH). This work was funded by the University of Wisconsin-Madison MRSEC under grant numbers DMR 0520527 and DMR 0079983.

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Appendix

A1. *Alternate procedure for contacting LC with lipid vesicles*

In an effort to minimize the presence of droplets of LC in the aqueous dispersion of vesicles, LC (5CB or TL205) and an aqueous dispersion of DPPC vesicles was gently and periodically swirled in order to facilitate contact between the two phases. Aliquots were removed from the aqueous phase, treated, and analyzed by UV–visible spectroscopy as described in the main body of the paper. It was apparent that the uptake of LC was slow and that equilibrium was not achieved after 31 days (figure A1). The values shown in figure A1 correspond to blank-subtracted average values, where the blank corresponds to phosphate buffer that had been contacted with LC at the same temperature.

A2. *Measurement of LC uptake into lipid vesicles above the melting transition temperature*

Using the procedures described above, we measured the uptake of TL205 or 5CB into vesicles of DPPC at 50°C as well as vesicles of DMPC at 37°C in order to evaluate the effect of lipid phase state (gel versus liquid crystalline/fluid) during uptake (figure A2). The results demonstrate that the uptake of 5CB into lipid vesicles in the fluid state occurred slightly more quickly than for lipid vesicles in the gel state. Importantly, the rate of uptake of 5CB was much greater than TL205, independent of the initial state of the lipid in the vesicle bilayer (gel or fluid).

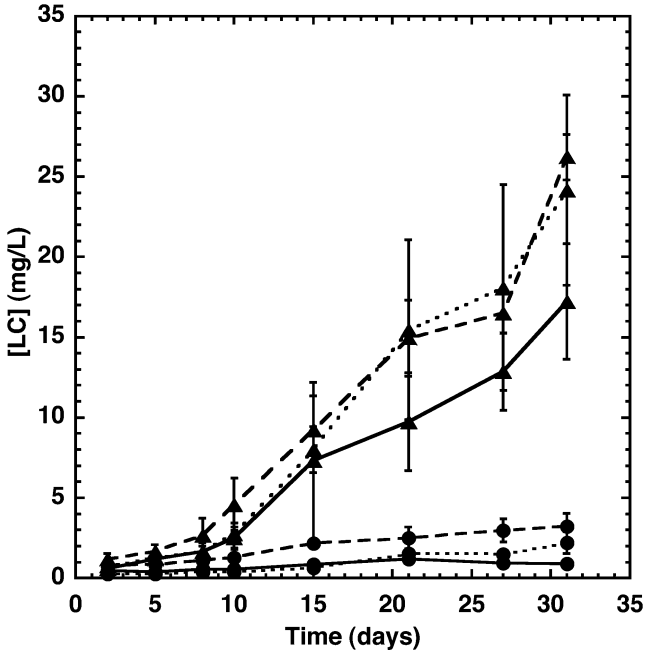

Figure A1. Uptake of 5CB (triangles) and TL205 (circles) into lipid vesicles of DPPC at 37°C (solid line), DPPC at 50°C (dashed line) and DMPC at 37°C (dotted line) as quantified by UV absorbance. Error bars correspond to plus or minus one standard deviation.

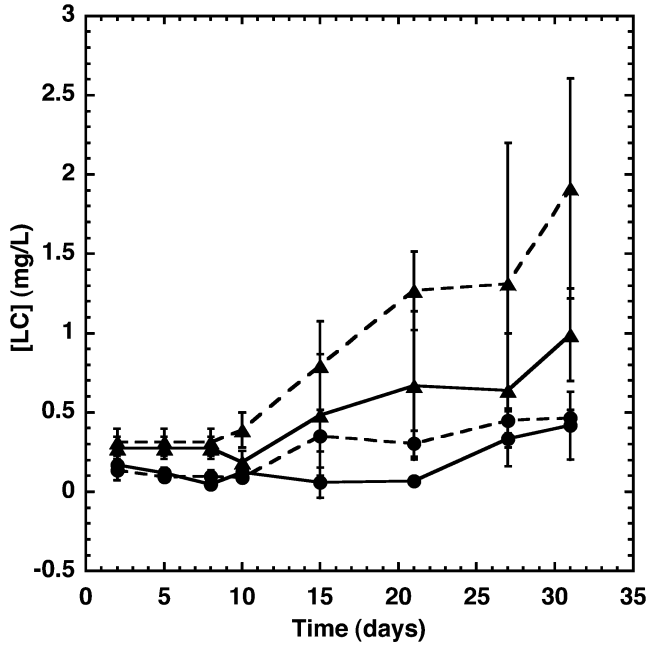

Figure A2. Solubility of 5CB (triangles) and TL205 (circles) into phosphate buffer (pH 7.4) at 37°C (solid line) and 50°C (dashed line) as quantified by UV absorbance. Error bars correspond to plus or minus one standard deviation.