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Amplification of the Stereochemistry of Biomolecular Adsorbates by Deracemization of Chiral Domains in Bent-Core Liquid Crystals

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Chirality is a key concept found in both chemistry and biology, with chiral asymmetries observed in many substances such as tartrates^[1] and biological helices, e.g., DNA, RNA, and polypeptide. Chirality in liquid crystals (LCs) is commonly induced by the presence of optically active chiral molecules, for instance, by doping non-chiral mesogens with chiral molecules. Such chiral LCs typically form helical suprastructures,^[2] which can be either one-dimensional (cholesteric, chiral smectic C, and twist grain boundary phases) or three-dimensional (blue phases).^[2] In this paper, we move to explore the general but challenging idea of using LCs to amplify biomolecular chirality at surfaces. The study was motivated by the proposal that achiral or racemic LCs, in which chirality is induced by external chiral stimuli such as a chiral surface,^[3] might, in the long-term, enable the design of new classes of biotic-abiotic interfaces in which the chirality of biomolecules is reported in the organization of the LCs. Herein we explore this idea using bent-core LCs (BCLCs, also known as banana-shaped liquid crystals).^[4,5] Macroscopic optical activity in the non-chiral BCLC systems has been observed in the B2,^[6] B4,^[7] and dark conglomerate (DC) phases.^[8] The B2 phase is a smectic phase with molecules tilted from the layer normal such that the chirality is defined by the relative orientation of the molecular long axis, the direction

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defined by the bent-core of the mesogen, and the smectic layer normal. Alternatively, and of relevance to the present study, the B4 phase is a so-called helical nanofilament phase, where molecules are tilted from the layer normal and form macroscopic chiral domains consisting of helical nanofilaments that exhibit significant optical activity.

Spontaneously resolved chiral domains in the B4 phase emerge with almost the same probability. The B4 phase, therefore, can be considered as a racemate. Similar to the technique of optical resolution used in organic synthesis, asymmetric induction resulting in finite enantiomeric excess (*ee*) is possible in the B4 phase by using various methods, e.g., the addition of chiral compounds,^[9,10] the use of an alignment layer with chiral side chains,^[11] irradiation by circularly polarized light,^[12] and the use of a twisted cell geometry.^[13] In the last two examples, the chiral external stimuli resulted in formation of macroscopic chiral domains with large *ee* (close to 100 %). Whereas the reports described above all deal with the B4 phase confined in a thin film between two solid surfaces, in the study reported in this paper, we move to investigate the B4 phase with interfaces to immiscible aqueous phases. As described in detail elsewhere, aqueous interfaces to thermotropic LCs are a fundamentally interesting class of interfaces because biological molecules can be introduced into the aqueous phase, and their self-assembly at the aqueous-LC interface can lead to surface-driven ordering transitions in the LCs.^[14–19] For example, past studies have reported that formation of protein-phospholipid complexes at aqueous interfaces of nematic LCs can trigger anchoring transitions in LCs that are easily transduced using polarized light transmitted through the LCs. In addition, because the mobilities of adsorbates are high at aqueous-LC interfaces (as compared to LC-solid interfaces), past studies have also observed that LCs can influence the organization of adsorbates at aqueous-LC interfaces (e.g., change the phase behavior of lipid monolayers). In this paper, we move beyond these past studies to investigate the influence of chirality of biological adsorbates on the organization of LCs at aqueous-LC interfaces where the LC is a bent core LC. Specifically, we sought to determine if phospholipids adsorbed at the isotropic 12OAz5AzO12 (Scheme 1)/solution interface might act as chiral triggers that nucleate large biased chiral domains as the B4 phase is formed upon cooling.

We used phospholipids spontaneously adsorbed onto the interface between a LC film of 12OAz5AzO12 and an aqueous glycerol solution (hereafter abbreviated as LC/solution interface). The bent-shaped dimer 12OAz5AzO12 (Scheme 1) exhibits the phase sequence; Iso - SmC_A - B4. In samples confined between two glass slides with 5.5 μm spacers (so-called sandwich cells), the B4 domains were almost optically isotropic and appeared dark under crossed polarizers (Figure 1, center). As shown in the right and left micrographs in Figure 1, however, the two domains were distinguished as dark and bright domains under de-crossed polarizers, indicating spontaneous segregation into chiral domains with opposite handedness or chirality.

Next we investigated the influence of phospholipid molecules adsorbed at the interface between an aqueous solution and the bent-core material. We hypothesized that the helical sense (helicity) of the nanofilaments of the B4 phase would be predetermined by the chiral sense (chirality) of the phospholipids located at the interface when the isotropic phase is cooled into the B4 phase via the SmC_A phase. In these experiments, the bent-shaped dimer 12OAz5AzO12 was filled within the pores of a 5-μm-thick gold grid supported on an octadecyltrichlorosilane (OTS)-treated glass slide which anchored the LC normal to the surface. When the sample was heated in air (Figure 2A and B), below the Iso-SmC_A transition temperature (112 °C), we observed a typical schlieren texture of SmC_A between crossed polarizers (Figure S1A). Upon cooling further into the B4 phase, the LC film segregated into domains with (+) or (–) chirality with almost equal probability, as confirmed by using de-crossed polarizers (Figure 3A). As compared to typical sandwich cells (Figure

1), the texture of the B4 phase in the grid with a free surface was comprised of domains exhibiting a higher birefringence. Next, we immersed the film into an aqueous glycerol solution (free of phospholipid), heated it to 120 °C (above the isotropization temperature; Figure 2C), and then slowly cooled it to room temperature at a rate of 0.5 °C/min. The texture of the B4 phase in this experiment, as shown in Figure 3B, differed significantly from that shown in Figure 3A, consistent with the influence of a change in interfacial ordering of the LC. However, similar to Figure 3A, the segregation of chiral domains is still evident in Figure 3B.

We investigated the influence on the B4 phase of two phospholipids that possessed opposite chirality, *L*-DLPC (*L*- α -dilauroyl phosphatidylcholine) and *D*-DPPC (*D*- α -dipalmitoyl phosphatidylcholine). As shown in Figure 2C, one of the phospholipids was added into an aqueous glycerol solution, and the B4 film (Figure 2B) was immersed into the solution. When incubated at room temperature, the B4 films showed no obvious ordering transitions after being immersed for a week in aqueous glycerol solutions containing the phospholipids. From this result, we conclude that any adsorption of the phospholipids onto the interface of the B4 phase does not change the ordering of the 12OAz5AzO12 in the B4 phase. Hence, we heated the 12OAz5AzO12 into its isotropic phase (120 °C) for 10 min and subsequently cooled it slowly to room temperature (Figure 2C and D). When viewed using polarizing optical microscopy (POM), as shown in Figure 3C for *L*-DLPC, counterclockwise (left) and clockwise (right) rotations of the analyzer led to differences in the optical appearance (darker and brighter, respectively) of the sample that were consistent with biased chiral domain formation.

In order to confirm asymmetric chirality induction, we performed circular dichroism (CD) measurements. Figure 4 shows (A) CD spectra obtained using a grid cell of 12OAz5AzO12 and (B) a UV-visible absorption spectrum of a chloroform solution of 12OAz5AzO12. After immersing the grid cell into the aqueous glycerol containing *L*-DLPC, heating into the isotropic phase (Figure 2C) and then cooling to the B4 phase (Figure 2D), the grid cell exhibited a clear positive CD peak at the absorption maximum (360 nm). In contrast, *D*-DPPC-adsorbed B4 films showed a negative CD peak. The signs of the optical activity of the B4 phase correlate, therefore, with those of the phospholipids. These results support our hypothesis that phospholipids adsorbed at the LC/solvent interface serve as a chiral trigger when helical nanofilaments grow at the SmC_A-B4 transition. This interpretation is also supported by the past study of Shiromo et al.,^[11] in which they observed films of chiral polyimides supported on solid surfaces in sandwich cells to induce an enantiomeric excess of 10% in the chiral domains of the B4 phase. Significantly, whereas the study reported above showed that a chiral surface alignment layer at a solid surface can induce an enantiomeric excess of chiral domains of 10%, our study reveals that spontaneous adsorption of a chiral amphiphile from an *aqueous* phase can trigger a *complete* deracemization (close to 100% *ee*) in the achiral BCLC film. That is, once homochiral helical nanofilaments start to form from the aqueous interface of the bent-core mesogens, chirality-preserving growth^[20] makes the bulk homochiral.

We interpret the asymmetric chirality induction described above to be based on the effect of phospholipids at the LC/solution interface, i.e., chiral-interface-induced asymmetric chirality in the bulk. Specifically, we propose that phospholipids exist only at the interface and not in the bulk (as in other methods of chiral doping^[9,10]). Time-of-flight secondary ion mass spectroscopy (ToF-SIMS), an effective and powerful method for chemical analysis of the uppermost layer in thin films, was chosen as a technique that could provide information on the molecular composition of the phospholipid-decorated film. Figure 5A shows a spectrum (mass range $m/z = 621\text{--}624$) of the surface region (within 1 nm of the dry interface) of the B4 phase that had been incubated against *L*-DLPC. The mass spectra over a full mass range

($m/z = 0-800$) are shown in Figure S3. Additional experimental details can also be found in the SI and the experimental section. The characteristic peaks in Figure 5A are due to the fragments that originate exclusively from a specific component of the interfacial structure, that is, *L*-DLPC. On the other hand, the peaks corresponding to the mass of *L*-DLPC were absent from the spectrum after the surface of the sample was etched by electron beam sputtering (Figure 5B). The sputtering removed a layer of material that was approximately a few hundred nanometers in thickness. These two results support our conclusion that the phospholipids exist only at the surface of the film and that the influence of the phospholipids on the ordering of the B4 phase is mediated via interactions of phospholipids and mesogens at the interface of the B4 phase.

In conclusion, we have succeeded in inducing asymmetric chirality to the B4 phase consisting of helical nanofilaments by using chiral phospholipids adsorbed spontaneously from an aqueous/glycerol solution. Asymmetric chirality induction was confirmed by means of POM and CD measurements. According to CD spectroscopy, *L*-DLPC- and *D*-DPPC-adsorbed B4 phases exhibited unichiral domains of opposite handedness. The spectra of ToF-SIMS confirmed that the phospholipids are present only at the interface of the BCLC film, thus supporting our proposition that they influence the chirality of the B4 phase via interactions mediated at the interface of the LC phase. Overall, these results suggest that BCLCs provide a class of materials that can be used to amplify molecular chirality into macroscopic phenomena. The results also provide guidance for the design of LC materials for the creation of biotic-abiotic interfaces.

Experimental

Materials

Bent-shaped dimer, α , ω -bis(4-alkoxyazobenzene-40-carbonyloxy)alkene (12OAz5AzO12, Scheme 1A), was synthesized according to Niori et al.^[21] and Izumi^[22] (DIC corporation, non-commercial product). The phase sequence and the transition temperatures measured by differential scanning calorimetry (DSC) were Iso (112 °C) SmC_A (99 °C) B4 on cooling and B4 (105 °C) SmC_A (113 °C) Iso on heating. The phase transition between the two mesogenic phases obtained was 5 °C higher than that previously reported.^[12]

L- α -dilauroyl phosphatidylcholine (*L*-DLPC, Scheme 1B) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Octadecyltrichlorosilane (OTS), *D*- α -dipalmitoyl phosphatidylcholine (*D*-DPPC, Scheme 1C), and glycerol were purchased from Sigma-Aldrich (St. Louis, MO). Gold specimen grids (5 μ m thickness, 50 μ m wide bars, and 283 μ m grid) were purchased from Electron Microscopy Sciences (Fort Washington, PA). Glass slides were obtained from Fisher Scientific (Pittsburgh, PA).

Sample Preparation

In the present study, sample films were prepared as follows. Detailed descriptions used to clean glass slides and to coat OTS layers on the slides can be found in previous publications.^[14,15,23,24] About 3 mg of 12OAz5AzO12 was placed on the OTS-coated slide and heated to 120 °C above the clearing point (112 °C) using a hot stage (FP82HT, Mettler) and a controller (Central Processor FP90, Mettler). Next, a gold specimen grid was set on top of the 12OAz5AzO12, filling the squares of the gold grid with melted 12OAz5AzO12 (Figure 2A). A glass capillary tube was used to remove excess compound above the grid. The quality of the OTS-treated layers was confirmed by observing the alignment of nematic LC (5CB) introduced between two OTS-coated slides.

Adsorption of Phospholipid Molecules on the LC films

To prepare solutions into which LC samples were immersed, the following procedure was adopted. As amphiphilic molecules, chiral phospholipids (either *L*-DLPC or *D*-DPPC with a concentration of 2 mM) were used. *D*-DPPC, which was obtained in a powder form, was dissolved in chloroform to a known concentration. Next, the chloroform was evaporated under a stream of nitrogen gas, resulting in dried-*D*-DPPC. The dried film of *D*-DPPC was subsequently dissolved into water. For *L*-DLPC, which was purchased in liquid form (at 10 mg/mL chloroform), the *L*-DLPC was dried and then dissolved in water. The aqueous solutions containing phospholipids was mixed for 10 s by vortexing and sonication, alternately. This stirring process was repeated 3 times. Then glycerol was added into the aqueous solution to prepare an aqueous glycerol solution with 80 wt % of glycerol with a final phospholipid concentration of 2 mM.

An LC film within a gold grid was placed onto the bottom of shallow glass dish at room temperature (in the B4 phase), and then the aqueous glycerol solution with phospholipid molecules was added into the dish. After incubating the dish containing the LC film for 5 h at room temperature, the dish was heated to 120 °C, and was kept for 10 min. Next, the dish was slowly cooled down to room temperature at a rate of 0.5 °C/min. For circular dichroism (CD) and Time-of-Flight Secondary Ion Mass spectra (ToF-SIMS) measurements, the LC film was taken out of the solution (Figure 2D). The films were rinsed with ethanol and dried under a stream of nitrogen gas.

Measurements

Spontaneous chiral segregation in the B4 phase of the bent-shaped dimer was observed by using a polarizing optical microscope (Optical Microscopy BX60, Olympus) in transmission mode. LC films immersed in an aqueous solution or sandwich cells were placed on a rotating stage. Chiral segregation was established by a differential change in the transmittance of light through the domains upon $\pm 10^\circ$ rotation of the analyzer.

The chiral properties of LC films with adsorbed lipid molecules were investigated by CD spectroscopy (Spectrometer J-720WI and Spectropolarimeter Power Supply PS-450J, JASCO) and absorption measurement (UV/VIS Spectrometer, JASCO V-560).

Positive and negative ToF-SIMS were measured for the *L*-DLPC-adsorbed B4 films (Primary ion: Bi_3^{++} , Sputtering ion: O_2 ; TOF-SIMS 5–100-AD, Ion-TOF GmbH). This measurement probes a spot size of 0.1 mm² and a depth of ~1 nm for each spectrum obtained. By using this technique, we can confirm that phospholipids are localized to the interface and not dissolved in the bulk (i.e., bulk chiral dopant). In our measurements, the B4 film was removed from the phospholipid solution, rinsed with ethanol, and dried under a stream of N_2 prior to performing ToF-SIMS measurements. The peaks in the negative spectra appeared to be generally less specific; therefore, only the positive secondary-ion intensities were used for the evaluation of the relationship between SIMS intensities and lipid adsorption. Prominent peaks in the mass range ($m/z = 1\text{--}800$) were unambiguously assigned on the basis of the comparison between experimental and theoretical masses of the secondary species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Polarizing optical micrographs of 12OAz5AzO12 in the B4 phase between glass substrates (5.5 μm thick). The arrows indicate the directions of polarizer and analyzer. The analyzer was rotated ± 10 degrees from the crossed position in the micrographs on the left and the right, respectively.

Figure 2.

Schematic illustrations of phospholipid molecules adsorbed on the LC/solution interface. (A) A gold grid was filled with isotropic 12OAz5AzO12. (B) The isotropic 12OAz5AzO12 was cooled slowly to room temperature to form the B4 phase. (+) and (–) indicate domains of opposite chirality that form spontaneously during the transition to the B4 phase. (C) The LC film that was immersed in an 80 wt % aqueous glycerol solution containing phospholipids and was heated to the isotropic phase. (D) After cooling into the B4 phase, the film was removed from the solution for CD and ToF-SIMS measurements (see text and SI for details).

Figure 3.

Polarizing optical micrographs of films of the B4 phase of 12OAz5AzO12 supported within gold grids (5 μm thick). (A) Sample prepared in air. (B) Sample after immersion in an 80 wt % aqueous glycerol solution (free of lipid), after heating into the isotropic phase (120 $^{\circ}\text{C}$) and cooling back into the B4 phase (room temperature). (C) Sample after immersion in an 80 wt % aqueous glycerol solution with *L*-DLPC, after heating into the isotropic phase (120 $^{\circ}\text{C}$) and cooling into the B4 phase (room temperature). The observations in (B) and (C) were made when the grid cell was immersed under the aqueous solution of glycerol.

Figure 4. CD and UV-visible absorption spectra of 12OAz5AzO12. (A) CD spectra of the B4 phase of 12OAz5AzO12 with interfaces decorated with phospholipid molecules (either L-DLPC or D-DPPC). (B) Absorption spectrum of a chloroform solution containing 20 μ M 12OAz5AzO12.

Figure 5.

Positive secondary ion spectrum at the top-surface of (A) a sample of the B4 phase of 12OAz5AzO12 that was decorated with *L*-DLPC, and (B) the same sample after sputtering of the surface of the sample. The mass spectra are shown in the mass range $m/z = 621$ –624.