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Ordering Transitions in Micrometer-Thick Films of Nematic Liquid Crystals Driven by Self-Assembly of Ganglioside GM₁

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Abstract

We report an investigation of the self-assembly of the monosialoganglioside (GM₁) at interfaces formed between aqueous solutions of 10 μ M GM₁ (at 25°C) and micrometer-thick films of the nematic liquid crystal (LC) 4'-pentyl-4-cyanobiphenyl (5CB). We observe the process of spontaneous transfer of GM₁ onto the interfaces to be accompanied by continuous ordering transitions within the micrometer-thick films of the LC. At saturation coverage, the GM₁ orders the LC in an orientation that is perpendicular to the interface, an orientation that is similar to that caused by phospholipids such as dilaurylphosphatidylcholine (DLPC). This result suggests an interaction between the LC and GM₁ that is dominated by the hydrophobic tails of the GM₁. Relative to DLPC, however, we observe the dynamics of the LC ordering transition driven by GM₁ to be slow (2 hours for DLPC versus 100 hours for GM₁). To provide insight into the origins of the slow dynamics of the GM₁-induced ordering transition in the LC, we performed two additional measurements. First, we quantified the time-dependent adsorption of GM₁ at the LC interface by using fluorescently-labeled GM₁. Second, we used the Langmuir-Schaefer method to transfer preorganized monolayers of GM₁ from an air-water interface to the aqueous-LC interface. Results obtained from these two experiments are consistent with a physical picture in which the final stages of spontaneous adsorption/ordering of GM₁ at the aqueous-LC interface dictate the dynamics of the LC ordering transition. This rate limiting process underlying the ordering transition was substantially accelerated by heating the system above the phase transition temperature of GM₁ (26 °C), suggesting that the phase state of the GM₁ micellar aggregates in bulk solution strongly influences the kinetics of the final stages of ordering/adsorption of GM₁ at the LC interface. Overall, these results and others presented in this manuscript reveal that it is possible to decorated interfaces of a nematic LC with GM₁, and that the assembly of GM₁ at these interfaces impacts the dynamic and equilibrium ordering of the LC.

Keywords

Ganglioside; GM₁; Self-Assembly; Liquid Crystals; Biomolecular Interfaces; Langmuir-Schaefer; Ordering Transitions; Anchoring of Liquid Crystals; Biosensors

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INTRODUCTION

A series of recent studies have reported on ordering transitions induced in micrometer-thick films of liquid crystals (LC) that are caused by the self-assembly of amphiphiles at aqueous interfaces of LC films.^{1–13} Of the various different amphiphiles explored in these studies (which includes surfactants, phospholipids, macromolecular amphiphiles), the ordering of nematic LCs by the interfacial self-assembly of the phospholipid $L\text{-}\alpha$ -dilauroylphosphatidylcholine (L -DLPC) has been particularly well characterized.^{3, 5, 6, 9–13} Specifically, equilibration of aqueous dispersions of unilamellar vesicles formed from L -DLPC has been shown to lead to formation of monolayers of DLPC at the interfaces of the LC. The formation of the monolayer of L -DLPC has in turn been demonstrated to cause the LC to adopt a perpendicular (homeotropic) ordering at the aqueous interface. Additional experiments have also revealed that (i) the phase behavior of monolayers of DLPC formed at the interfaces of the nematic LC is substantially different from that observed in the absence of the nematic ordering of the LC, and (ii) that binding of proteins (such as phospholipases) to these lipid-laden LC interfaces leads to easily visualized ordering transitions in the LCs.^{5, 6} Whereas an increasingly complete understanding of the assembly of phospholipids at aqueous interfaces of LCs is emerging, in this paper, we move to report on the assembly of glycolipids at aqueous-LC interfaces. In particular, as a prototypical example of the wide range of glycolipids found in biological systems, we focus on the self-assembly of the monosialoganglioside GalBeta1-3GalNAcBeta1-4(NeuAcAlpha2-3)GalBeta1-4GlcBeta1-1'-ceramide (GM_1) at aqueous interfaces of thermotropic LCs. We note that GM_1 binds the bacterial toxin produced by *Vibrio cholerae* and, consequently, that the biophysical properties of GM_1 have been studied in detail within Langmuir monolayers and lipid bilayers.

Gangliosides, in general, are lipids with head groups comprised of oligosaccharides containing one or more N-acetylneuraminic acid (sialic acid) residues.¹⁴ GM_1 contains a pentasaccharide (Figure 1). As noted above, the self-organization of gangliosides within *in vitro* mimics of biological membranes have been widely studied^{15–22} as have the interactions of protein toxins with these models of cell membranes.^{23–35} In the context of the former investigations, the physicochemical characteristics of GM_1 -laden interfaces have been investigated with atomic force microscopy (AFM),^{16, 19–21, 24} light scattering techniques,^{17, 18} surface plasmon resonance (SPR),²⁵ and fluorescence microscopy.³⁶ In this paper, we report the results of an investigation that sought to create a new class of GM_1 -decorated interfaces that are prepared by self-assembling GM_1 at the interfaces of LCs. By analogy to phospholipid-decorated LCs, we hypothesized that the orientational ordering of the LC would be closely coupled to the formation of the GM_1 -decorated interface, and thus that the LC ordering behavior could be used to report on the interfacial behavior of the GM_1 . Although certain similarities in the orientational ordering of the LC in the presence of phospholipids and GM_1 are noted in our paper, the results of our study also reveal striking differences in the dynamics of these two systems. We end our introduction by noting that the development of methods that lead to formation of GM_1 -laden interfaces of LCs, and an understanding of the equilibrium and dynamic properties of the interfaces, is a prerequisite to exploring their potential use as biomolecular interfaces at which protein toxin interactions with GM_1 can be reported via ordering transitions in LCs. In future studies, we will investigate the influence of protein toxins on the ordering of GM_1 -decorated interfaces of LCs.

MATERIALS & METHODS

Materials

GalBeta1-3GalNAcBeta1-4(NeuAcAlpha2-3)GalBeta1-4GlcBeta1-1'-Cer (GM_1) was obtained from Avanti Polar Lipids, Inc.(Alabaster, AL). Trizma-hydrochloride (Tris [hydroxymethyl]aminomethane hydrochloride, Tris HCl), sodium azide,

ethylenediaminetetraacetic acid (EDTA), and chloroform were obtained from Sigma-Aldrich (St. Louis, MO). Octadecyltrichlorosilane (OTS), sodium chloride, methanol, methylene chloride, sulfuric acid, hydrogen peroxide (30% w/v), 2-propanol, and heptane were obtained from Fisher Scientific (Pittsburgh, PA). BODIPY FL C₅-GalBeta1-3GalNAcBeta1-4 (NeuAcAlpha2-3)GalBeta1-4GlcBeta1-1'-Cer (BODIPY FL-GM₁) was purchased from Molecular Probes (Eugene, OR). Sodium hydroxide was obtained from LabChem Inc. (Pittsburgh, PA). The LC 4'-pentyl-4-cyanobiphenyl (5CB) was obtained from EM Sciences (New York, NY). All chemicals were used as obtained. Deionization of a distilled water source was performed with a Milli-Q system (Millipore, Bedford, MA) to give water with a resistivity of 18.2 MΩ•cm. Glass microscope slides were Fisher's Finest Premium Grade obtained from Fisher Scientific. Gold specimen grids (20 μm thickness, 50 μm wide bars, and 283 μm grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA).

Preparation of LC-filled grids

Detailed descriptions of procedures used to prepare LC-filled grids can be found in our previous publications.^{1-13, 37} Briefly, glass microscope slides were cleaned according to published procedures³⁸ and coated with OTS.⁸ The quality of the OTS layer was assessed by checking the alignment of 5CB confined between two OTS-coated glass slides. Any surface not causing homeotropic anchoring (perpendicular alignment of 5CB) of 5CB was discarded.^{39, 40} Gold specimen grids that were cleaned sequentially in methylene chloride, ethanol, and methanol were placed onto the surface of OTS-coated glass slides. Approximately 1 μL of 5CB was dispensed onto each grid and then excess LC was removed by contacting a capillary tube with the droplet of 5CB.^{1-13, 37} We estimated the variation in 5CB thickness to be approx ±10% based on interference colors generated when viewing the 5CB with white light. Each LC-filled grid was equilibrated at ambient temperature and subsequently immersed in or contacted with the aqueous solution of interest at the desired temperature.

Preparation of Aqueous Dispersions of GM₁

Dispersions of GM₁ were prepared following published procedures.^{5, 9, 32, 35} Briefly, GM₁ was dissolved in a mixture of chloroform and methanol (4:1 by volume) and dispensed into glass vials. The chloroform:methanol mixture was evaporated under a stream of N₂, and the vial containing the lipids was then placed under vacuum for at least 2 hours. The dried lipid was resuspended in an aqueous solution consisting of 50 mM Tris HCl, 200 mM NaCl, 3 mM NaN₃, and 1 mM Na₂EDTA adjusted using NaOH to a pH of 7.5 (denoted as pH 7.5 TRIS buffer in the remainder of this paper). This procedure resulted in a slightly cloudy solution. Before contacting with LC interfaces, the aqueous dispersion of GM₁ was equilibrated at ambient temperatures for at least 1 hour. Experiments utilizing the aqueous GM₁ dispersions were typically initiated within 4 hours of their preparation.

Determination of the Orientation of Liquid Crystals by Polarized Light Microscopy

The orientation of 5CB was observed by using an Olympus BX60 microscope with crossed polarizers (transmission mode). LC-filled grids were placed on a rotating stage located between the polarizers. Orthoscopic examinations were performed with the source light intensity set to 50% of full illumination and the aperture set to 10% to collimate the incident light. Homeotropic (perpendicular) alignment of a LC was determined by first observing the absence of transmitted light during a 360° rotation of the sample. Next, insertion of a condenser below the stage and a Bertrand lens above the stage allowed conoscopic examination of the specimen. An interference pattern consisting of two crossed isogyres confirmed the homeotropic alignment.⁴¹ Images were captured with a microscope-mounted digital camera (Olympus C-4000 Zoom) set to an f-stop of 2.8 and a shutter speed of 1/320 s. Temperature was controlled with a heat

stage (INTEC, Inc., Boulder, CO) or a water bath (ISOTEMP 202, Fisher Scientific, Pittsburgh, PA).

Epifluorescence Imaging of GM₁-Laden Aqueous-LC Interfaces

Lipid layers comprised of mixtures of BODIPY FL-GM₁ and GM₁ were formed by incubating the LC interface against aqueous dispersions of GM₁ that contained 1 mol% BODIPY FL-GM₁. Before performing the epifluorescence measurements, the LC-filled grid was sequentially moved from the aqueous dispersion of BODIPY FL-GM₁ and GM₁ to 2 mL and then subsequently 400 mL of the pH 7.5 TRIS buffer (see above for composition) before a final transfer into a third volume of the pH 7.5 TRIS buffer solution. During each transfer of the sample between solutions, care was taken to ensure that a thin film of the water covered the lipid-laden interface of the 5CB hosted within the grid.^{4, 42} GM₁-laden interfaces were then imaged by epifluorescence microscopy using an Olympus IX71 inverted microscope equipped with a 100 W mercury lamp. A fluorescence filter cube with excitation at 480 nm and emission at 535 nm (Chroma, Rockingham, VT) was used to visualize BODIPY FL fluorescence. Images were collected with a Hamamatsu 1394 ORCA-ER CCD camera (Bridgewater, NJ) connected to a computer and controlled through SimplePCI imaging software (Compix, Inc., Cranberry Twp., NJ).

Fluorescence imaging was performed using an objective power of 4X and an exposure time of 0.3 seconds. After each epifluorescence measurement, the sample was discarded. At least 4 replicate LC-filled grids were measured at each time point. To account for effects of fluorophore degradation/bleaching encountered when using long incubation times (>72 hours), we normalized all fluorescence measurements to the intensity measured using an OTS-treated slide immersed in the same aqueous dispersion of BODIPY FL-GM₁/GM₁. All fluorescence intensity measurements were corrected for background fluorescence, measured using lipid-free buffer solutions. Intensity values were determined using SimplePCI and Adobe Photoshop.

Quantification of Interfacial Concentration of BODIPY FL-GM₁/GM₁ Adsorbed at Aqueous-LC Interfaces via Fluorimetric Measurements

The interfacial concentration of BODIPY FL-GM₁/GM₁ adsorbed at the aqueous-LC interface was determined by using the following procedure. First, a calibration plot of fluorimetric intensity versus known BODIPY FL-GM₁ concentration in bulk solution prepared using 2 mL of a 2-propanol solution containing 1 μ L of 5CB, known amounts of BODIPY FL-GM₁ and GM₁ (at molar ratio of BODIPY FL-GM₁ to GM₁ of 1:99), and 5 μ L of pH 7.5 TRIS buffer solution. This matrix was used so as to mimic the solution composition that resulted when BODIPY FL-GM₁/GM₁-decorated 5CB hosted within grids was extracted and then dissolved in a 2-propanol solvent (see below). Fluorimetric measurements were performed using a FluoroMax-3 fluorimeter (Instruments S. A/Jobin Yvon/Spex Horiba Group, Edison, NJ) with an excitation wavelength of 480 nm (0.5 nm excitation slit) and an emission wavelength range of 490–850 nm (5 nm emission slit) for the detection of BODIPY fluorescence. The fluorimeter was connected to a computer and controlled using DATAMAX software (Instruments S. A./Jobin Yvon/Spex Horiba Group).

To determine the interfacial concentration of BODIPY FL-GM₁/GM₁ adsorbed at an aqueous-LC interface, the LC sample (LC hosted in grid, volume of LC was $\sim$ 1 μ L) was physically transferred from the aqueous BODIPY FL-GM₁/GM₁ dispersion to 2 mL and then 400 mL of pH 7.5 TRIS buffer solution to successively dilute BODIPY FL-GM₁/GM₁ in the bulk solution contacting the surface of the LC sample. Each sample was then removed from the pH 7.5 TRIS buffer, and a blunt-tip 10 μ L micro-syringe was used to withdraw the lipid-decorated 5CB from each grid. The 5CB extracted using the micro-syringe was dissolved in 2 mL 2-propanol for fluorimetric measurements to determine the concentration of BODIPY FL-GM₁ in the

sample. We illuminated the solutions at 480 nm and recorded the emission intensity from 490 to 850 nm to ensure that only monomer emission was observed with a peak around 510 nm. With a known BODIPY FL-GM₁ concentration versus fluorimetric intensity calibration plot (Figure S1), this measurement permitted quantification of the interfacial concentration of GM₁ adsorbed at the interface as a function of time during the LC ordering transition. Polarized light microscopy was used to confirm that all LC was extracted from each grid. Control experiments not containing BODIPY FL-GM₁ were performed to determine the background fluorescence. Furthermore, to ensure that fluorescence intensities resulted only from assemblies of BODIPY FL-GM₁/GM₁ adsorbed at the aqueous-5CB interface (and did not include any contributions from the fluorophore-containing bulk solution), we confirmed that the 400 mL pH7.5 TRIS buffer solution used to dilute excess BODIPY FL-GM₁/GM₁ at the LC sample surface did not possess significant fluorescence intensity after the last dilution step (data not shown). As noted above, we observed the fluorimetric intensity of BODIPY-FL in the bulk aqueous solution to decrease in experiments that involved long incubation times (>72 hours) due to photobleaching/degradation. To correct for this process of degradation, fluorimetric measurements of the bulk solution intensity were recorded at each time point. All raw fluorimetric intensities measured after ~ 72-hour of incubation were corrected for this process of degradation. To minimize degradation processes, light exposure was minimized in all steps of the experiments.

The above measurements, when combined with knowledge of the areas of the aqueous-LC interfaces within each grid, were used to calculate the areal density (area/molecule) of the GM₁ molecules assembled at each interface. All experiments were repeated 4 times using the same batch of reagents (e.g., 2-propanol, BODIPY FL-GM₁, and GM₁ from the same lot number). Error bars were calculated as the standard deviation of these replicates. As described in our Results section, the above-described procedure for quantification of the interfacial concentration of lipid was validated by determining the interfacial concentration of L-DLPC at saturation coverage (for which values have been reported in the literature).

Preparation of GM₁ Monolayers at Aqueous-LC Interfaces by using the Langmuir-Schaefer Method

Langmuir monolayers of GM₁ were prepared using a NIMA 602A film balance (Coventry, England) equipped with a paper Wilhelmy plate for surface pressure measurements. A 4:1 v/v chloroform:methanol solution of GM₁ (~0.3 mg/mL) was spread at the aqueous-air (pH 7.5 TRIS buffer) interface at 25 °C. Temperature was controlled with a water bath (ISOTEMP 1006D, Fisher Scientific, Pittsburgh, PA). The solvent was allowed to evaporate for 20 minutes at 25 °C before monolayer compression was initiated. Symmetric monolayer compression was performed at a rate of 100 cm²/min (26.0 ~ 34.6 Å²/molecule·min). Once the desired surface pressure was reached, the LC sample was lowered horizontally into contact with the monolayer at the aqueous-air interface using tweezers and immediately submerged into the pH 7.5 TRIS buffer sub-phase for analysis using polarized microscopy. Detailed descriptions regarding the experimental setup used for the Langmuir-Schaefer transfer can be found elsewhere.¹⁰

RESULTS and DISCUSSION

Spontaneous Adsorption of GM₁ onto Aqueous-LC Interfaces

Our initial experiments sought to determine if GM would adsorb spontaneously from bulk aqueous solution onto the interface of a LC and thereby trigger an ordering transition in the LC. We also sought to determine whether or not the dynamic and equilibrium characteristics of LC ordering transitions triggered by GM₁, if observed, would be similar to those reported previously for phospholipids such as L-DLPC.^{3, 5, 6, 9, 11, 12} To this end, we first contacted nematic 5CB hosted within grids (see Materials and Methods for details) with aqueous

dispersions of 10 μM GM₁ (pH 7.5 TRIS buffer) at 25 °C. As shown in Figures 2A–H, we recorded the optical appearance of the LC under crossed polars for 336 hours (14 days). Immediately following contact with the aqueous dispersion of GM₁, the optical appearance of the LC appeared colorful and bright (Figure 2A). This optical appearance of the LC is caused by orientations of the LC that are illustrated in the cartoon shown in Figure 3A. In brief, as described in past studies,^{3, 5, 6, 9, 11, 12} in the absence of adsorbates at the aqueous-LC interface, the orientation of 5CB is close to parallel to the aqueous-LC interface. At the interface of the OTS-treated glass, the LC assumes a perpendicular orientation. These two boundary conditions, when combined, result in the introduction of splay and bend distortions into the LC as the film of LC accommodates the two boundary conditions. The LC,^{2, 4} when viewed under white light illumination, possesses pale yellow-green and pink hues due to interference effects caused by the birefringence of the LC. Variation in the azimuthal orientation of the 5CB results in the dark brush patterns evident in Figure 2A within each compartment of the grids.

Inspection of Figures 2A–H reveals that the optical appearance of the LC changed gradually from bright to dark during incubation against the aqueous dispersion of 10 μM GM₁ for ~ 240 hours. Orthoscopic and conoscopic examination of the sample shown in Figure 2F (see Methods for procedures) indicated that the LC had slowly transitioned to a homeotropic orientation at the aqueous-LC interface during the 240 hours (see Figure 3B for a schematic illustration). To further characterize the nature of the transition, we used the interference colors observed under white light illumination to calculate the tilt angle of the LC (orientation of the LC at the aqueous-LC interface measured with respect to the surface normal, with 0° corresponding to homeotropic alignment; detailed descriptions of methods can be obtained from our past study⁴) during incubation against the aqueous dispersion of GM₁ (Figure 3C). Inspection of Figure 3C reveals that the change in the orientation of the LC occurred in two steps. Within the first 24 hours, the tilt of the LC changed to 65°, and subsequently, between 100 hours and 200 hours, a second continuous transition in the orientation to the homeotropic state occurred. Incubation of the LC against the aqueous dispersion of GM₁ for times longer than 240 hours did not result in any further changes in the orientation of the LC (see Figures 2F–H, and Figure 3C).

We interpret the results described above to indicate that GM₁ does transfer spontaneously from aqueous dispersions onto the aqueous-LC interface, and that the GM₁ present at the aqueous-LC interface does cause an ordering transition in the LC. The final state of the interface corresponds to the homeotropic orientation of the LC. Although the final homeotropic state of the GM₁-laden interface of the LC is similar to that observed in past studies of the orientation of phospholipid-decorated interfaces of LCs^{5, 9} and thus suggests that the hydrophobic tails of the GM₁ play a central role in defining the interactions of GM₁ with the nematic LC, we note here two important differences between the behaviors of GM₁ and phospholipids. First, past studies of the self-assembly of L-DLPC (10 μM in 10mM Tris (hydroxymethyl)-aminomethane and 100 mM NaCl at pH 8.9, referred hereafter to as pH 8.9 TBS buffer) at the aqueous-LC interface have reported that L-DLPC will trigger an ordering transition of 5CB within 2 hours.^{5, 9} In contrast, the dynamics of the 5CB ordering transition induced by contact with the dispersion of GM₁ (Figures 2A–H) was surprising slow. Second, past observations of LC ordering transitions driven by L-DLPC have revealed that it can occur through a process that involves the nucleation and growth of domains of oriented LC: In contrast, from inspection of Figures 2A–H, it is evident that the ordering transition driven by GM₁ is a continuous one.⁹ We note, however, that the above-described measurements with GM₁ and L-DLPC were performed in different aqueous buffers.

In order to provide a more precise comparison of the characteristics of the ordering transitions of the LC induced by GM₁ and L-DLPC, we performed measurements of the ordering of

nematic 5CB incubated against aqueous dispersions of L-DLPC prepared using the same buffer as used in our measurements with GM_1 (pH 7.5 TRIS buffer; Figures 2A–H). Optical micrographs of the LC incubated against the L-DLPC are shown in Figures 2I–P, and the tilt angle of the LC at the aqueous-LC interface is shown in Figure 3D. We make two principal observations from these measurements. First, the time-scale of the ordering transition induced by L-DLPC when dispersed in pH 7.5 TRIS buffer is much shorter (~ 4 hours) than that observed with GM_1 (240 hours). Second, consistent with past measurements of the self-assembly of L-DLPC at the aqueous-LC interface from pH 8.9 TBS buffer^{5, 9} the ordering transition of the LC induced by L-DLPC in TRIS pH 7.5 buffer involves domains of oriented LC. Figure S3 of Supporting Information presents a direct comparison of the ordering transition of the LC induced by L-DLPC dissolved in the two buffers. Inspection of this Figure S3 reveals relatively minor effects of the buffer, the most pronounced effect being a change in the rate of the ordering transition (the rate changes by a factor of approximately 2).

The most striking difference between the behavior of GM_1 and L-DLPC that emerges from the above-reported study is the slower dynamics of the ordering transition induced by GM_1 , as compared to L-DLPC . To provide a first perspective on the possible origins of the slow ordering transition induced by GM_1 , we calculated the time for GM_1 molecules present in bulk solution at a concentration of $10 \mu\text{M}$ to diffuse to the aqueous-LC interface in quantity sufficient to saturate the interface with GM_1 molecules. The calculated diffusion time was ~ 4 minutes, estimated as $L^2/2D_m$ where $L = \Gamma/C$ and corresponds to the diffusion distance, D_m is the diffusion coefficient of GM_1 aggregates (micelles) obtained from light scattering measurements⁴³ ($D_m = 3.7 \times 10^{-6} \text{ cm}^2/\text{s}$), Γ is the surface concentration of GM_1 molecules at the interface, and C is the concentration of GM_1 in the bulk solution. For this calculation, we estimated the density of GM_1 molecules at the aqueous-LC interface to be $\sim 40 \text{ \AA}^2/\text{GM}_1$.²⁶ We note that the conclusion extracted from this calculation is not sensitive to the exact value of the interfacial density that we assumed. The main conclusion is that the ordering transition of 5CB driven by GM_1 is not controlled by diffusion-limited transport of GM_1 to the interface.

Quantification of the Interfacial Concentration of BODIPY FL- GM_1/GM_1 at Aqueous-LC Interfaces via Fluorescence Measurements

We hypothesized that the slow ordering transition of the LC induced by spontaneous adsorption of GM_1 from the bulk aqueous dispersion onto the aqueous-LC interface (~ 240 hours) was due to slow kinetics of adsorption of GM_1 from the bulk dispersion adjacent to the interface. We note that GM_1 dispersed under the aqueous solution conditions used in our study is known to form micellar aggregates, and evolution of the aggregate structure and size distribution has been reported to occur slowly (over days) at room temperature^{43, 44}. To test the above-described hypothesis, we incorporated 1% BODIPY FL- GM_1 (Figure 1) into a mixture of BODIPY FL- GM_1 and GM_1 , and used epifluorescence intensity measurements, as described in the Methods section, to record the rise in fluorescence of the aqueous-LC interfaces at various time intervals following contact with the BODIPY FL- GM_1/GM_1 . Inspection of Figure 4A confirms BODIPY FL- GM_1/GM_1 adsorption at the interface of the LC. Surprisingly, however, inspection of Figure 4A suggests that the interfacial concentration of the mixture of BODIPY FL- GM_1 and GM_1 reached near-saturation coverage (within experimental uncertainty) within ~ 20 hours. In contrast, as noted in Figure 2 and Figure 3, polarized light microscopy revealed the ordering transition of the LC to occur over ~ 240 hours following contact with the dispersion of GM_1 . In particular, inspection of Figure 3C shows that the orientation of the LC was nearly parallel to the interface after ~ 20 hours of incubation in the aqueous dispersion of GM_1 .

Although the above-described measurements lead to the suggestion that the ordering transition of the LC lags substantially behind the adsorption of the GM_1 onto the aqueous-LC interface, quantitative interpretation of fluorescence intensity measurements during adsorption processes

should be interpreted cautiously because past studies have shown that BODIPY-labeled lipids possess concentration-dependent emission properties that are consistent with dimer formation in both monolayer^{45, 46} and unilamellar vesicle^{47, 48} systems. For example, Dahim *et al* have demonstrated that if the BODIPY monomers are sufficiently close (critical radius is 25.9 ± 1.8 Å) within a fluid monolayer at the time of excitation, excited dimers of BODIPY will form and the monomer emission at 515 nm will be progressively replaced by the dimer emission at ~ 620 nm.⁴⁵ To address possible artifacts associated with dimer formation in the epifluorescence measurements shown in Figure 4A, we pursued a second methodology based on fluorescence measurements wherein the lipids adsorbed at the aqueous-LC interface were extracted, and quantified after dissolution into an organic solvent (see Methods for the details). This method had two advantageous attributes. First, dilution of BODIPY FL-GM₁ into the organic solvent avoided the potential for dimer formation. Second, the methodology led to an estimate of the *absolute density* of BODIPY FL-GM₁ molecules at the aqueous-LC interface.

To validate the second methodology, we first used it to quantify the interfacial concentration of L-DLPC within monolayers adsorbed at the aqueous-5CB interface, as determined previously by Brake and coworkers^{4-6, 9} (see Supporting Information). The fluorimetric results revealed that at saturation coverage, the areal density of the L-DLPC at the interface was 48 ± 10 Å²/L-DLPC. This value is in reasonable agreement with the area occupied by L-DLPC at the air-water interface within condensed monolayers.^{10, 49-53} In addition, recent experiments by Meli and coworkers used the Langmuir-Schaefer method to transfer monolayers of L-DLPC from the aqueous-air interface onto the aqueous-5CB interface. These experiments revealed that monolayers of L-DLPC with an area density between 45 and 56 Å²/L-DLPC molecule caused homeotropic ordering of L-DLPC (consistent with our experimental results).¹⁰

Figure 4B shows a plot of the fluorimetric intensity of mixtures of BODIPY FL-GM₁ and GM₁ extracted from the aqueous-LC interface, as a function of time of incubation of the LC against the GM₁ dispersion (at 25 °C). As can be seen in Figure 4B, the fluorimetric intensity of the extracted BODIPY FL-GM₁/GM₁ reached a saturation value within 24 hours of incubation. This result is consistent with epifluorescence measurements shown in Figure 4A, suggesting that the saturation of the epifluorescence intensity in Figure 4A was not an artifact associated with dimer formation. We note that the uncertainties in the values of the fluorescence intensity shown in Figure 4B are substantial, limiting the precision with which we can define the time at which saturation coverage of the interface takes place. We suspect that variation in the shape of the LC-aqueous meniscus leads to variation in the LC interfacial area, and thus capacity of the interface to adsorb the lipid. By combining the calibration plot shown in Figure S1 with the mean value of the fluorimetric intensity shown in Figure 4B in the limit of long adsorption times, we calculate the limiting area density of GM₁ at the aqueous-LC interface to be 50 ± 12 Å²/GM₁. This mean area density is similar to the density of GM₁ at the air-aqueous interface at which surface pressure-area isotherms indicate densely-packed, condensed monolayers of GM₁ to form.^{17, 54-56} Past studies have reported that GM₁ forms a densely-packed monolayer at $\sim 50-62$ Å²/GM₁ molecule and a tilted, condensed monolayer at $\sim 62-71$ Å²/GM₁ molecule within Langmuir films (26 °C and a subphase pH of 5.6),⁵⁶ following the definitions described by Kaganer *et al.*⁵⁷

The results above lead us to conclude that a relatively high surface concentration of GM₁ (52 ± 14 Å²/GM₁) at the aqueous-LC interface is achieved within ~ 24 hours of placement of the LC into contact with the aqueous dispersion of GM₁; further incubation of the LC against the GM₁ dispersion does not result in a measurable increase in the surface concentration of GM₁ (50 ± 12 Å²/GM₁), whereas the ordering of the LCs changes over 9 days of subsequent incubation. The above described observations led us to consider several possible physical scenarios. First, we considered it possible that the slow ordering transition of the LC was triggered by the very final stages of adsorption of the GM₁, which leads to changes in surface

concentrations of GM₁ that lie beyond the precision of the methods we have developed to determine the surface concentrations. Second, we considered it possible that the *adsorbed* GM₁ undergoes a slow ordering process at the interface, and that an ordered state of the GM₁ triggers the ordering transition in the LC. Below, we report experiments that were performed to distinguish between these two scenarios.

GM₁ Adsorption and LC Anchoring Transitions at the Aqueous-LC Interface

The first experiment we performed sought to find evidence for the second physical scenario, in which ordering of the *adsorbed* GM₁ controls the rate of the LC ordering transition. To this end, we incubated the LC against an aqueous dispersion of 10 μ M GM₁ at 25 °C for 24 hours and then physically transferred the LC into a lipid-free buffer solution. Through transfer of the GM₁-laden aqueous-5CB interface into the pH 7.5 TRIS buffer, we eliminated the possibility of additional GM₁ adsorption. We hypothesized that if the slow ordering of the LC was due to a process that involved the ordering of adsorbed GM₁ at the interface, the ordering transition of the LC in the above experiment would be similar to that shown in Figure 2. It was not. After transfer of the GM₁-decorated LC interface to the lipid-free buffer, we observed the LC to remain in an orientation that was nearly parallel to the interface (at 25°C) for the subsequent ~240 hours. This result suggests that additional adsorption of GM₁ to the interface of the LC occurs during the prolonged incubation of the LC interface against the dispersion of GM₁, and that the final stages of the adsorption process leading to saturation of the GM₁-laden interface is a slow process that regulates the dynamics of the LC ordering transition.

The above-mentioned results suggest that the rate of adsorption of GM₁ is much lower than for L-DLPC. We note that past studies of gangliosides and phospholipids have demonstrated that they differ substantially in their self-assembly behavior in bulk solution and at interfaces. The sialic acid moiety of the head group of GM₁ gives the ganglioside a negative charge near neutral pH.^{14, 58} The resulting electrostatic interactions influence the phase behavior of GM₁ both in aqueous dispersions and in monolayers formed at air-water interfaces (as evidenced in surface pressure-area isotherms and measurements of bulk phase transition temperatures (T_m , from a gel phase to a fluid phase).⁵⁹ When compared with DPPC (with two saturated C₁₆ chains),⁶⁰ dilauroylphosphatidylcholine (DLPC, with two saturated C₁₂ chains),^{10, 49–53} and dioleoylphosphatidylcholine (DOPC, with two symmetric unsaturated C₁₈ chains),⁶¹ the surface pressure-area isotherm of GM₁ has a larger collapse pressure and the liquid expanded (LE)/liquid condensed(LCD) phase coexistence region is observed at higher surface pressure.⁵⁴ The higher collapse and LE/LCD phase coexistence pressures of the GM₁ isotherm suggests that intermolecular GM₁ interactions within a well-packed monolayer are more repulsive than with phospholipids, and thus the kinetics of adsorption of GM₁ at the aqueous-LC interface may be slower than for DLPC and other zwitterionic phospholipids.

Langmuir-Schaefer Transfer of Monolayers of GM₁ from the Aqueous-Air Interface to the Aqueous-LC Interface

The results above suggest that the slow ordering transition of the LC induced by GM₁ is due to a slow rate of adsorption of GM₁ to the aqueous-LC interface during spontaneous transfer from an aqueous dispersion of GM₁. To further test this proposition, we hypothesized that processes that would lead to accelerated rates of transfer of GM₁ onto the aqueous-LC interface would lead to more rapid ordering transitions of the LC. Here we report the use of the Langmuir-Schaefer transfer technique, as described recently by Meli *et al*,^{10, 11} to rapidly transfer preformed, high density monolayers of GM₁ onto the aqueous interface of the LC. Figure 5A shows the surface pressure-area isotherm for GM₁ within a Langmuir monolayer, with data points indicating the areal densities of GM₁ at which monolayers of GM₁ were transferred to the interface of the LC. Figures 5B–J show optical micrographs (crossed polars) of the LC after transfer of the preformed GM₁ monolayers onto the interface of the LC. Inspection of Figures

5B–E reveals that liquid-condensed monolayers of GM₁ ($< 53 \text{ \AA}^2/\text{GM}_1$ molecule) cause homeotropic ordering of the LC within 1 min of transfer onto the interface of the LC. Tilted states of the LC were observed when the density of GM₁ at the interface of the LC was between $53\text{--}58 \text{ \AA}^2/\text{GM}_1$ (Figures 5F and G). Finally, liquid-condensed/liquid-expanded monolayers of GM₁ ($> 58 \text{ \AA}^2/\text{GM}_1$ molecule) resulted in near-planar alignment of the LC. Because homeotropic ordering of the LC was observed almost immediately following transfer of the preformed densely-packed monolayer of GM₁ onto the LC, these results support our hypothesis that the slow ordering transition of the LC observed during spontaneous adsorption of GM₁ is due to a slow adsorption process leading to completion of the GM₁ monolayer.

Influence of Temperature on the Adsorption of GM₁ to Aqueous-LC Interfaces

Past studies have demonstrated by using differential scanning calorimetry (DSC) measurements that hydrated GM₁ undergoes a broad endothermic phase transition at $26 \text{ }^\circ\text{C}$ ^{59, 62, 63} In multilayer systems formed by GM₁ below the phase transition temperature, the mobility of the GM₁ was low. Above the phase transition temperature, the tails of the GM₁ showed increased degrees of freedom, as is typically for the so-called L_α phase.⁶⁴ Because the measurements described above lead to the conclusion that the slow ordering transition of GM₁ is due to a slow adsorption process, and given that our measurements were performed at $25 \text{ }^\circ\text{C}$ (below the above-mentioned phase transition temperature of GM₁), we investigated the influence of temperature on GM₁ adsorption at the LC interface. We sought to determine if the phase state of GM₁ in the aqueous dispersions played a central role in the slow adsorption process that we noted above. Figure 6 reveals that the temperature of the GM₁ dispersion does play a key role in the dynamics of the ordering transition. Specifically, we observed a threshold time that characterized the planar-to-homeotropic ordering transition (defined as the time required for LC within at least 6 of 40 grid squares in the Au grid to turn homeotropic) decreased dramatically above 25°C (Figure 6). We further note that the liquid crystal 5CB is nematic at 25°C , and that it undergoes a bulk phase transition from a nematic phase to an isotropic phase at $35 \text{ }^\circ\text{C}$.^{65, 66} Because of this phase transition, the data reported in Figure 6 at temperatures above 35°C was obtained by cooling the 5CB to room temperature just before observation of the orientation of the LC. Overall, the results in Figure 6 strongly support our proposition that the phase state of GM₁ plays a central role in the rate of adsorption of GM₁, and thus the dynamics of the ordering transition of the LC. This result is consistent with past reports of the adsorption of phospholipids. Whereas L-DLPC ($T_m = -1 \text{ }^\circ\text{C}$) caused nematic 5CB to undergo an ordering transition from planar to homeotropic (vesicle dispersion in pH 8.9 TBS buffer) within ~ 2 hours, DPPC ($T_m = 41 \text{ }^\circ\text{C}$) (in pH 8.9 TBS buffer) did not spontaneously form densely packed monolayers at interfaces of LCs within the experimental times investigated (days).^{5, 9} In this context, the dynamics of the anchoring transition of 5CB upon contact with GM₁ were relatively slow compared to L-DLPC , but faster than that of pure DPPC. We conclude that the differing rates of the LC ordering transitions induced by L-DLPC , DPPC, and GM₁ correlate closely with the phase states of the lipids within the lipid vesicles/micelles.^{5, 9}

CONCLUSIONS

The principal conclusions of this paper are threefold. First, by using polarized light microscopy, we have demonstrated that self-assembly of the glycolipid GM₁ at the aqueous-LC interface triggers an anchoring transition in the LC that results in homeotropic ordering of the LC. Relative to ordering transitions induced by L-DLPC , however, the dynamics of the LC transition induced by GM₁ are very slow. Second, although both epifluorescence microscopy and fluorimetric measurements show that a relatively high surface concentration of GM₁ ($52 \pm 14 \text{ \AA}^2/\text{GM}_1$) at the aqueous-LC interface is achieved within 24 hours of contact with aqueous dispersions of GM₁, our results suggest that a slow process that leads to completion of the

GM₁ interfacial assembly must take place in order to trigger the ordering transition in the LC. This conclusion is supported by experiments in which the Langmuir-Schaefer technique is used to rapidly transfer preformed monolayers of GM₁ to the aqueous-LC interface: rapid delivery of a densely-packed GM₁ monolayer ($<53\text{\AA}^2/\text{GM}_1$) of GM₁ to the aqueous-LC interface led to an almost immediate transition of the LC to the homeotropic orientation. Third, we demonstrate the time-dependent ordering transitions of LCs induced by GM₁ are strongly influenced by the phase state of GM₁ within micelles in aqueous dispersions. At temperatures above approximately 30°C, the LC ordering transitions induced by GM₁ take places on a time-scale comparable to that observed when using L-DLPC. Overall, the measurements reported in this paper provide a quantitative characterization of the dynamic and equilibrium characteristics of ordering transitions induced by GM₁-decorated aqueous-LC interfaces. These results provide a foundation of knowledge that can be used to guide the design of biomolecular interfaces based on LCs at which interactions involving GM₁ can be studied.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ABBREVIATIONS

5CB, 4'-pentyl-4-cyanobiphenyl; L-DLPC, L- α -dilauroylphosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-Glycero-3-phosphocholine; GM₁, monosialoganglioside GalBeta1-3GalNAcBeta1-4(NeuAcAlpha2-3)GalBeta1-4GlcBeta1-1'-Ceramide; BODIPY FL-GM₁, BODIPY FL- GalBeta1-3GalNAcBeta1-4(NeuAcAlpha2-3)GalBeta1-4GlcBeta1-1'-Cer..

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REFERENCES

1. Kinsinger MI, Sun B, Abbott NL, Lynn DM. *Advanced Materials* 2007;19:4208–4212.
2. Lockwood NA, de Pablo JJ, Abbott NL. *Langmuir* 2005;21:6805–6814. [PubMed: 16008390]
3. Lockwood NA, Abbott NL. *Current Opinion In Colloid & Interface Science* 2005;10:111–120.
4. Brake JM, Mezera AD, Abbott NL. *Langmuir* 2003;19:8629–8637.
5. Brake JM, Daschner MK, Luk YY, Abbott NL. *Science* 2003;302:2094–2097. [PubMed: 14684814]
6. Brake JM, Abbott NL. *Langmuir* 2007;23:8497–8507. [PubMed: 17595119]
7. Brake JM, Mezera AD, Abbott NL. *Langmuir* 2003;19:6436–6442.
8. Brake JM, Abbott NL. *Langmuir* 2002;18:6101–6109.
9. Brake JM, Daschner MK, Abbott NL. *Langmuir* 2005;21:2218–2228. [PubMed: 15752009]
10. Meli MV, Lin IH, Abbott NL. *Journal of American Chemical society* 2008;130(13):4326–4333.
11. Gupta JK, Meli MV, Teren S, Abbott NL. *Physical Review Letters* 2008;100:048301. [PubMed: 18352339]
12. Lockwood NA, Gupta JK, Abbott NL. *Surface Science Reports* 2008;63:255–293.
13. Lockwood NA, Meli MV, Surjosantoso A, Kim EB, de Pablo JJ, Abbott NL. *Liquid Crystals* 2007;34(12):1387–1396.
14. Cox, N. *Principles of Biochemistry*. New York: W. H. Freeman and Company; 2005.
15. Maggio B, Cumar FA, Caputto R. *Biochem. J* 1978;175:1113. [PubMed: 743230]

16. Vie V, Mau NV, Lesniewska E, Goudonnet JP, Heitz F, Grimellec CL. *Langmuir* 1998;14:4574–4583.
17. Majewski J, Kuhl TL, Kjaer K, Smith GS. *Biophysical Journal* 2001;81:2707–2715. [PubMed: 11606283]
18. Hirai M, Iwase H, Hayakawa T, Koizumi M, Takahashi H. *Biophysical Journal* 2003;85:1600–1610. [PubMed: 12944276]
19. Yokoyama S, Ohta Y, Sakai H, Abe M. *Colloids and Surfaces B: Biointerfaces* 2004;34:65–68.
20. Wang R, Shi J, Parikh AN, Shreve AP, Chen L, Swanson BI. *Colloids and Surfaces B: Biointerfaces* 2004;33:45–51.
21. Ohta Y, Yokoyama S, Sakai H, Abe M. *Colloids and Surfaces B: Biointerfaces* 2004;33:191–197.
22. Brown RE. *Journal of Cell Science* 1998;111:1–9. [PubMed: 9394007]
23. van Heyningen S. *Science* 1974;183:656–657. [PubMed: 4810267]
24. Luckham PF, Smith K. *Faraday Discuss* 1998;111:307–320. [PubMed: 10822616]
25. Kuziemko GM, Stroh M, Stevens RC. *Biochemistry* 1996;35:6375–6384. [PubMed: 8639583]
26. Miller CE, Majewski J, Faller R, Satija S, Kuhl TL. *Biophysical Journal* 2004;86:3700–3708. [PubMed: 15189866]
27. Turnbull WB, Precious BL, Homans SW. *Journal of American Chemical Society* 2004;126:1047–1054.
28. Vengasandra S, Sethumadhavan G, Yan F, Wang R. *Langmuir* 2003;19:10940–10946.
29. Lencer WI, Hirst TR, Holmes RK. *Biochimica et Biophysica Acta* 1999;1450:177–190. [PubMed: 10395933]
30. Picking WL, Moon H, Wu H, Picking WD. *Biochimica et Biophysica Acta* 1995;1247:65–73. [PubMed: 7873593]
31. Miller IR, Vinkler H, Yavin E. *Bioelectrochemistry and Bioenergetics* 1989;22:365–377.
32. Schon A, Freire E. *Biochemistry* 1989;28:5019–5024. [PubMed: 2765522]
33. Ribí HO, Ludwig DS, Mercer KL, Schoolnik GK, Kornberg RD. *Science* 1988;239:1272. [PubMed: 3344432]
34. Wisniewski BJ, Bramhall JS. *Nature* 1981;289:319–321. [PubMed: 6256663]
35. Fishman PH, Moss J, Osborne JJC. *Biochemistry* 1978;17:711–716. [PubMed: 623739]
36. Fang Y, Frutos AG, Lahiri J. *Langmuir* 2003;19:1500–1505.
37. Park JS, Abbott NL. *Advanced Materials* 2008;20:1185–1190.
38. Skaife JJ, Abbott NL. *Chem. Mater* 1999;11:612–623.
39. Cognard J. *Mol. Cryst. Liq Cryst* 1982;1:1.
40. Yang JY, Mathauer K, Frank CW. *Microchemistry: Spectroscopy and Chemistry in Small Domains* 1994:441.
41. Bloss FD. *An Introduction to the Methods of Optical Crystallography*. 1961
42. Cremer PS, Boxer SG. *J. Phys. Chem* 1999;103(13):2554–2559.
43. Corti M, Cantu L, Salina P. *Advances in Colloid and Interface Science* 1991;36:153–171. [PubMed: 1910627]
44. Orthaber D, Glatter O. *Chemistry and Physics of Lipids* 1998;92:53–62. [PubMed: 9631537]
45. Dahim M, Mizuno NK, Li XM, Momsen WE, Momsen MM, Brockman HL. *Biophysical Journal* 2002;83(3):1511–1524. [PubMed: 12202376]
46. Mikhalyov I, Gretskeya N, Bergstrom F, Johansson LBA. *Phys. chem. Chem. Phys* 2002;4:5663–5670.
47. Pagano RE, Martin OC, Kang HC, Haugland RP. *J. Cell Biol* 1991;113:1267–1279. [PubMed: 2045412]
48. Tleugabulova D, Zhang Z, Brennan JD. *J. Phys. Chem. b* 2002;106:13133–13138.
49. Phang TL, Liao YC, Franses EI. *Langmuir* 2004;20(10):4004–4010. [PubMed: 15969391]
50. Kim S, Yu H. *J. Phys. Chem* 1992;96(10):4034–4040.
51. Tamada K, Kim S, Yu H. *Langmuir* 1993;9(6):1545–1550.
52. Thuren T, Virtanen JA, Kinnunen PKJ. *Chemistry and Physics of Lipids* 1990;53:129–139.

53. Pinazo A, Infante MR, Park SY, Franses EI. *Colloids and Surfaces B: Biointerfaces* 1996;8:1–11.
54. Gomez-Serranillos IR, Minones J, Dynarowicz-Latka P, Iribarnegaray E, Casas M. *Colloids and Surfaces B: Biointerfaces* 2005;41:63–72.
55. Rosetti CM, Oliveria RG, Maggio B. *Langmuir* 2003;19(2):377–387.
56. Luckham P, Wood J, Froggatt S, Swart R. *Journal of Colloid and Interface Science* 1993;156:164–172.
57. Kaganer VM, Mohwald H, Dutta P. *Reviews of Modern Physics* 1999;71(3):779–819.
58. Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, JD. *Molecular biology of the cell*. New York & Landon: Garland Publishing Inc.; 1994.
59. Maggio B, Ariga T, Sturtevant JM, Yu RK. *Biochemistry* 1985;24:1084–1092. [PubMed: 4096890]
60. Kuhnau U, Madler B, Wurlitzer S, Rapp G, Schmiedel H. *Mol Cryst. Liq Cryst* 1997;304:171–178.
61. Bohorquez M, Patterson LK. *Langmuir* 1990;6:1739–1742.
62. Reed RA, Shipley GG. *Biophys. J* 1996;70:1363. [PubMed: 8785291]
63. Curatolo W, Small DM, Shipley GG. *Biochimica et Biophysica Acta-Biomembranes* 1977;468:11.
64. Garti, N. *Thermal behavior of dispersed systems*. Marcel Dekker Inc.; New York: 2001.
65. Collings, PJ.; Hird, M. *Introduction to liquid crystals*. Philadelphia: Taylor & Francis Ltd; 1997.
66. Luk YY, Campbell SF, Abbott NL, Murphy CJ. *Liquid crystals* 2004;31:611.

Figure 1.
 Structures of GM₁, BODIPY FL GM₁, DLPC and DPPC. Gal = Galactose, Glc = Glucose,
 GalNAc = N-Acetylgalactosamine, Neu5Ac = N-Acetylneuraminic acid.

Figure 2.

(A–P) Optical micrographs (crossed polars) of dynamic LC ordering transitions triggered by spontaneous adsorption of (A–H) GM₁ and (I–P) L-DLPC from aqueous solution at 25°C. Times are indicated in units of hours, and $t = 0$ hrs corresponds to the time of contact of the 10 μM GM₁ or 10 μM DLPC aqueous dispersion with the interface of the nematic 5CB. The GM₁ and DLPC dispersions were prepared in pH 7.5 TRIS buffer. Scale bar is 300 μm .

Figure 3.

(A) Schematic illustration corresponding to Figure 2A and 2I, showing the orientation of the nematic LC the aqueous-LC and OTS-LC interfaces, and the deformation of the LC induced by these boundary conditions. (B) Schematic illustration corresponding to Figure 2H and 2P, showing the homeotropic orientation of the LC at the interface of the LC decorated with GM₁ or DLPC. (C and D) Effective tilt angles of 5CB at the aqueous-LC interface estimated from interference colors generated by the 5CB with white-light illumination. The tilt angles are plotted as a function of time of adsorption of (C) GM₁ and (D) DLPC, with each lipid present at a concentration of 10 μ M in pH 7.5 TRIS buffer.

Figure 4.

Quantification of BODIPY FL-GM₁/GM₁ adsorbed at aqueous-5CB interfaces by fluorescence measurements at 25 °C (10 μ M GM₁ in pH 7.5 TRIS buffer). (A) Plot of epifluorescence intensity generated by mixed BODIPY FL-GM₁/GM₁ assemblies adsorbed at the interface of nematic 5CB as a function of time. (B) Plot of fluorimetric intensity generated by mixed BODIPY FL-GM₁/GM₁ assemblies adsorbed at the LC interface, and subsequently dissolved into 2-propanol for quantification. (C) Plot of the corresponding area per GM₁ molecule at the LC interface as a function of time, obtained using data shown in part B.

Figure 5.

(A) Surface pressure-area isotherm measured at 25°C for a Langmuir film of GM₁ formed on an aqueous subphase comprising pH 7.5 TRIS buffer. Squares depict surface pressure-area conditions under which Langmuir-Schaefer transfer to the LC interface was performed. (B to J) Polarized light micrographs of nematic 5CB after Langmuir-Schaefer transfer of GM₁ monolayers (prepared at the indicated surface pressure) to the interface of the LC. Scale bar is 300 μm.

Figure 6.

(A) Time required for GM₁ adsorption to trigger an ordering transition in nematic 5CB, plotted as a function of the temperature of the system. The GM₁ was adsorbed from a 10 μ M GM₁ dispersion in pH 7.5 TRIS buffer. (B) Expanded view of A at the high incubation temperatures.