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Influence of droplet size, pH and ionic strength on endotoxin-triggered ordering transitions in liquid crystalline droplets

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Abstract

We report an investigation of ordering transitions that are induced in water-dispersed, micrometer-sized droplets of a thermotropic liquid crystal (LC) by the bacterial lipopolysaccharide endotoxin. We reveal that the ordering transitions induced by endotoxin – from a bipolar state of the droplets to a radial state – are strongly dependent on the size of the LC droplets. Specifically, as the diameters of the LC droplets increase from 2 μm to above 10 μm (in phosphate buffered saline with an ionic strength of 90 mM and a pH of 7.2), we measured the percentage of droplets exhibiting a radial configuration in the presence of 100 pg/mL endotoxin to decrease from $98 \pm 1\%$ to $3 \pm 2\%$. In addition, we measured a decrease in either the ionic strength or pH of the aqueous phase to reduce the percentage of droplets exhibiting a radial configuration in the presence of endotoxin. These results, when interpreted within the context of a simple thermodynamic model that incorporates the contributions of elasticity and surface anchoring to the free energies of the LC droplets, lead us to conclude that (i) the elastic constant K_{24} plays a central role in determining the size-dependent response of the LC droplets to endotoxin, and (ii) endotoxin-triggered ordering transitions occur only under solution conditions (pH, ionic strength) where the combined contributions of elasticity and surface anchoring to the free energies of the bipolar and radial configurations of the LC droplets are similar in magnitude. Our analysis also suggests that the presence of endotoxin perturbs the free energies of the LC droplets by $\sim 10^{-17}$ J/droplet, which is comparable to the standard free energy of self-association of $\sim 10^3$ endotoxin molecules. These results, when combined with prior reports of localization of endotoxin at the center of LC droplets, are consistent with the hypothesis that self-assembly of endotoxin within micrometer-sized LC droplets provides the driving force for the ordering transitions. Overall, these results advance our understanding of ordering transitions triggered by the interactions of analytes with LC droplets and, more broadly, provide guidance to the design of LC droplet systems as the basis of stimuli-responsive soft materials.

Introduction

Past studies have established that the presence and organization of amphiphiles at interfaces formed between liquid crystals (LCs) and immiscible aqueous phases can influence the orientational ordering of the LCs.^{1, 2} For example, in systems containing LC confined to micrometer-sized droplets, it has been demonstrated that spontaneous adsorption of synthetic surfactants or lipids to the interfaces of the LC droplets can induce a bipolar-to-radial ordering transition, where the preferred alignment of the LC at the interface changes from planar (parallel, Fig. 1A) to homeotropic (perpendicular, Fig. 1B).³⁻⁶ These ordering transitions within LC droplets have been attributed to adsorbate-induced changes in the orientation-dependent part of the interfacial energy of the LCs (so-called anchoring

energy).³⁻⁶ To change the anchoring energy to induce such an ordering transition, a coverage of the interface by adsorbate of 0.1 to 1 Langmuir is typically required.^{3, 4, 7-9} More recently, however, we have reported on bipolar-to-radial ordering transitions within micrometer-sized droplets of nematic 4'-pentyl-4-cyanobiphenyl (5CB) dispersed in aqueous solutions that are driven by the presence of the bacterial lipopolysaccharide endotoxin at interfacial concentrations that are at least five orders of magnitude lower than the concentration required for saturation coverage (10^{-5} Langmuir).¹⁰ Such surface concentrations are well below those that lead to ordering transitions through uniform changes in anchoring energies.

The endotoxin used in the experiments reported above is comprised of a six-tailed glycopospholipid (called lipid A) in addition to two polysaccharide domains.¹¹ Several observations reported in our prior study hint at the origins of the endotoxin-triggered ordering transitions in the LC droplets.¹⁰ First, the ordering transitions induced by endotoxin at pg/mL concentrations were observed only when the LC was confined within micrometer-sized droplets. Specifically, ordering transitions were not observed when using planar, micrometer-thick films of 5CB (slab geometry) at comparable concentrations (pg/mL) of endotoxin. Second, the characteristic time-scales of the endotoxin-triggered ordering transitions were measured to be comparable to the times required for diffusion of endotoxin across the surfaces of the 5CB droplets, as would be required for localized association of the endotoxin on the droplet. Third, we observed pg/mL concentrations of endotoxin to induce the ordering transitions in the LC droplets through a kinetic pathway (series of transition states) that was different from that observed with adsorbates that induce changes in the anchoring energy of the LC droplets, thus providing further support for our conclusion that the ordering transitions in the LC droplets caused by pg/mL concentrations of endotoxin are not driven by uniform changes in anchoring energy.³⁻⁵ Finally, we demonstrated that endotoxin partitions to the center of LC droplets in the radial configuration (through the use of confocal microscopy with a fluorescently labelled endotoxin molecule), suggesting that endotoxin may be self-assembling at the locations of defects that form in LC droplets with radial configurations.¹²⁻¹⁴ While these past observations provide insight into the origins of endotoxin-triggered ordering transitions, a full understanding of the phenomenon is yet to be established. Herein we report an investigation that advances this understanding by examining the role of LC droplet size and aqueous solution conditions on the ordering transitions.

A number of past publications have reported analyses of the energetics of LC droplets by considering the equilibrium director configuration to be governed by surface anchoring and bulk elastic energies.¹⁴ When the easy axis of the LC is tangential to the droplet surface and the bulk elastic energy density is modelled using splay (K_{11}), twist (K_{22}), and bend (K_{33}) elastic constants¹⁵, the contributions of surface anchoring and bulk elastic energies to the overall free energy of the LC droplet stabilizes the bipolar configuration relative to the radial configuration, independent of droplet size. However, recent experimental and theoretical studies have also revealed the saddle-splay elastic constant (K_{24}) to play an important role in determining the equilibrium director configuration of LC droplets.^{6, 16, 17} For example, polymer-encapsulated 5CB droplets were observed to change from bipolar to radial configurations as the diameters of the droplets decreased, consistent with the effects of K_{24} .⁶ This result is important because it indicates that there exists a narrow range of LC droplet sizes in which K_{24} causes radial and bipolar droplets to be almost energetically degenerate (see below for additional details). As reported below, it is for LC droplets close to this size range that we observe endotoxin-driven ordering transitions in the pg/mL concentration range.

In this paper, motivated by the above considerations, we first report a series of experiments that investigated the influence of LC drop size on the endotoxin-triggered ordering transitions. These studies reveal a strong dependence on LC droplet size, consistent with the influence of K_{24} on the elastic energies of the LC droplets. In addition, we investigated the effects of changes in the ionic strength and pH of the aqueous phase in which the LC droplets were dispersed, which we interpret in terms of changes in anchoring energies.¹⁸ We place these results within the context of a simple thermodynamic model that leads to an estimate of the extent to which the association of endotoxin with LC droplets perturbs the energetics of the droplets.

Experimental

Materials

Endotoxin (from *E.coli* O127:B8) was purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (NaOH) and 85 wt% phosphoric acid (H_3PO_4) were obtained from Fisher Scientific (Pittsburgh, PA). The 4'-pentyl-4-cyanobiphenyl (5CB) was obtained from EM Sciences (New York, NY). Limulus amoebocyte lysate (LAL) reagent water was purchased from Associates of Cape Cod, Inc. (East Falmouth, MA). EndoTrap® Red Equilibration Buffer (described below as PBS buffer; 10mM Na_2HPO_4/NaH_2PO_4 , 80mM NaCl, pH7.2; certified to contain a concentration of endotoxin of less than 0.02 EU/mL (~2pg/mL)) and EndoTrap® Red 1/1 chromatography columns for endotoxin removal were purchased from Hyglos GmbH (Regensburg, Germany). Neptune pipette tips (no detectable endotoxin) were purchased from Continental Lab Product, Inc (San Diego, CA). Polystyrene tubes (certificated nonpyrogenic tubes) were purchased from Becton Dickinson Labware (Franklin Lakes, NJ). Fisher's Finest Premium Grade glass microscope slides and cover glass, and Wheaton glass 20mL scintillation vials were obtained from Fisher Scientific (Pittsburgh, PA). 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 $M\Omega \cdot cm$.

Preparation of LC emulsions

The emulsions were formed by sequential sonication and vortex mixing of 2 μL of nematic 5CB in 1mL of PBS at 25°C. Three cycles each comprising 10 seconds of vortex mixing (at 2500 rpm) and 10 seconds of sonication yielded milky white emulsions. The LC droplets were confirmed to exhibit bipolar configurations following sonication. The size distributions of the LC emulsion droplets were determined from optical micrographs using Image J (NIH, Bethesda, MD) software. The LC emulsions were observed visually to be stable against coalescence at least for 3 hours. The LC emulsions were used within 3 hours of preparation.

Preparation of buffers

PBS buffers of different ionic strength were prepared by diluting PBS with LAL reagent water. Following dilution, the PBS was mixed with a vortex mixer at 2500 rpm. Buffers of different pH were prepared by adjusting the pH of PBS with 1.0 and 0.1 M H_3PO_4 and NaOH solutions. The 1.0 and 0.1 M H_3PO_4 solutions were prepared by diluting the 85 wt% H_3PO_4 stock solution into Milli-Q water. The 1.0 and 0.1 M NaOH solutions were prepared by dissolving NaOH in Milli-Q water. After the pH adjustment and vortex mixing at 2500 rpm, the buffers were passed three times through an EndoTrap® Red 1/1 chromatography column to remove endotoxin.

Preparation of aqueous dispersions of endotoxin

Powdered endotoxin was dissolved in PBS at room temperature. After addition of the endotoxin powder to obtain a concentration of 20 $\mu g/mL$, each solution was mixed by

vortexing at 2500 rpm for 4 minutes. Serial dilutions were performed to reach a final concentration of either 10 pg/mL or 100 pg/mL. After dilution, each solution was vortexed for an additional 45 seconds. For experiments measuring the effect of ionic strength on the response of LC droplets to the presence of endotoxin, the final dilution step, to reach 10 pg/mL endotoxin, was performed in PBS of different ionic strengths. Similarly, for experiments measuring the effect of pH, the final dilution step, to reach 100 pg/mL endotoxin, was performed in PBS buffers of different pH.

Optical characterization of LC droplets by polarized light microscopy

A volume of 40 μL of an aqueous dispersion of endotoxin in PBS was dispensed onto a glass coverslip. A volume of 4 μL of an LC emulsion, prepared as described above, was added to the endotoxin dispersion to produce a sample containing 94,000 LC droplets. The ordering within the LC droplets was characterized 2 minutes after introducing the LC emulsion to the 100 pg/mL endotoxin solution to allow observation of larger droplets before the droplets sedimented to the bottom of the sample. The configurations of the LCs within the emulsion droplets were determined by observation of the droplets under an Olympus IX71 inverted microscope (Center Valley, PA) using an objective power of 100 $\times$ (an oil lens). Polarized light micrographs of the LC emulsions were collected with a Hamamatsu 1394 ORCAER CCD camera (Bridgewater, NJ) connected to a computer and controlled through SimplePCI imaging software (Compix, Inc., Cranberry Twp., NJ). As described in detail elsewhere, we characterized LC droplets that were at least 50 μm above the surface of the coverslip and translating with velocities greater than 1 $\mu\text{m/s}$ to avoid observation of droplets interacting with the surfaces of the coverslips.¹⁹ The droplets were diffusing (translating and rotating) – thus a radial droplet has an optical appearance that is invariant with time when viewed between crossed-polars, whereas the bipolar droplet has a distinct, time-varying optical appearance. Multiple images of each droplet were taken to determine the time-dependent optical texture of the droplet, and thus the director configuration.

Zeta potential measurements of LC droplets

Zeta potential measurements required a larger sample volume than optical characterization of the LC droplets. Therefore, 0.5 mL of an LC emulsion was added to 5 mL of PBS to maintain the 1:10 emulsion to sample ratio employed during optical characterization (see above). Following sample preparation, the zeta potential of the LC droplets was measured using a Malvern Instruments Zetasizer 3000 HSa (Malvern, Worcestershire, UK).

Results

In our first set of experiments, we investigated the influence of LC droplet size on the director configuration of droplets of 4'-pentyl-4-cyanobiphenyl (5CB) dispersed in an immiscible aqueous phase, both in the absence and presence of endotoxin. In the experiments reported below, we added to the aqueous phase a concentration of endotoxin of 100 pg/mL and characterized droplets with diameters ranging from 2 μm to 20 μm . If it is assumed that all of the endotoxin present in the aqueous solution associates with the LC droplets in our experiments, 100 pg/mL of endotoxin corresponds to roughly 10^3 endotoxin molecules per LC droplet (for additional details see *Size distribution of the LC droplets analyzed* in the ESI[†]). Furthermore, if the endotoxin was to adsorb to the surface of the LC droplets, a concentration of 100 pg/mL would lead to 10^{-5} of saturation coverage of the LC surfaces (saturation requires $\mu\text{g/mL}$ concentrations of an adsorbate^{3, 4, 7-10}).

[†]Electronic Supplementary Information (ESI) available: size distributions of LC droplets; size-dependent changes in LC configurations; methods for optical characterization; effects of pH and ionic strength; details regarding calculation of ionic strength. See DOI: 10.1039/b000000x/

Our experiments were initiated by emulsifying (see above for details) nematic 5CB in a phosphate buffered saline solution (PBS buffer), certified to have < 2 pg/mL of endotoxin. The PBS contained 10 mM sodium hydrogen phosphate (Na_2HPO_4), and 80 mM sodium chloride (NaCl) with a pH of 7.2. In each experiment, 4 μL of the 5CB emulsion was added to a 40 μL volume of 100 pg/mL endotoxin in PBS supported on a coverslip for observation.¹⁰ In the experiments reported below, we limited our observations to freely diffusing (rotating and translating) droplets that were located at least 50 μm above the surface of the coverslip to avoid the perturbing effects of interactions with the coverslip on the LC droplet configuration. These droplets possessed velocities of at least 1 $\mu\text{m/s}$.

We observed droplets with diameters larger than 2 μm to exhibit a bipolar configuration in the absence of endotoxin in solution.¹⁰ For example, Figs. 2A through 2D show bright field and polarized light micrographs of bipolar droplets with diameters of 6.5 ± 0.5 μm (Figs. 2A and 2B) and 10.5 ± 0.5 μm (Figs. 2C and 2D). A bipolar configuration is characterized by 5CB oriented tangentially to the droplet interface and two diametrically opposite surface point defects (called boojums) at the poles of the droplet (Fig. 1A).^{5, 20-22} Following the procedures employed in several previous reports, we identified droplets to be in a bipolar configuration by locating the boojums at the poles of the droplets (as indicated by the single-headed arrows in Fig. 2).^{5, 6, 10, 19} We emphasize that the droplets characterized in our study were diffusing (translating and rotating) in the aqueous solutions, resulting in a time varying optical appearance for droplets in the bipolar configuration (due to their lack of spherical symmetry).¹⁴ Therefore, the bright field and polarized light micrographs of bipolar droplets displayed in Fig. 2 represent one of many possible optical textures, and were selected to demonstrate the presence of both boojums. Boojums scatter light, and thus they can be easily identified in bright field images (Figs. 2A and 2C). The boojums can also be identified by their dark appearance when viewed between crossed-polars (Figs. 2B and 2D). Finally, we comment that under boundary conditions that impose a tangential alignment of LC at a droplet interface, two commonly observed director configurations are the bipolar and axial configurations.^{20, 23} It has been demonstrated that the bipolar configuration is favored over an axial configuration when the ratio of the bend elastic constant (K_{33}) to the splay elastic constant (K_{11}) exceeds unity ($K_{33}/K_{11} > 1$).²³ Therefore, our observation of the bipolar configuration in the absence of endotoxin is consistent with the values of K_{33}/K_{11} reported in literature for 5CB, which range from 1.3 to 1.6.²⁴⁻²⁶

In the presence of 100 pg/mL endotoxin, we observed a majority of LC droplets with diameters between 4 and 8 μm to undergo an ordering transition from a bipolar configuration to a configuration possessing radial (or close-to-radial, see below) symmetry, as characterized by optical microscopy. For example, Figs. 2E and 2F show bright field and polarized light micrographs of droplets with diameters of 6.5 ± 0.5 μm that exhibit a radial configuration (Fig. 1B).^{3-5, 13} In contrast to droplets in the bipolar configuration, droplets in the radial configuration exhibited an optical appearance that was invariant (as the droplets undergo rotational diffusion) over time.¹⁴ We make three additional comments regarding the radial configurations of the LC droplets. First, while the observations by optical microscopy revealed the configuration of the LC droplets to be radial, past simulation-based studies have reported that defects with various structures (e.g., point defect or disclination ring) can form at the centers of droplets that exhibit overall radial configurations.^{27, 28} The sizes of these defects, however, are smaller than the resolution of our microscope, and thus we do not resolve the structure of these defects in our measurements.^{29, 30} Second, consistent with previous studies, we observed many of the droplets in our experiments to exhibit optical appearances that indicated the presence of distorted radial configurations. This was particularly true for larger droplets (see ESI Fig. S2[†]).²⁷⁻³⁰ For example, when the diameters of the droplets exceeded 5 μm , we observed the extinction bands present in polarized light micrographs of several droplets to exhibit a slight twist relative to the orientation of the

crossed-polars, indicating the formation of twist deformations in the LC emanating from the centers of the droplets. Third, and most significantly, we observed that a majority of the LC droplets with diameters larger than 10 μm did not undergo an ordering transition in the presence of 100 pg/mL endotoxin (they remained in a bipolar configuration). Figs. 2G and 2H show bright field and polarized light micrographs of $10.5 \pm 0.5\text{-}\mu\text{m}$ -diameter 5CB droplets in the presence of 100 pg/mL endotoxin.

To provide insight into the origin of the key, third observation reported above, we quantified the percentage of droplets exhibiting the radial (or near-radial) configuration, in the presence of 100 pg/mL endotoxin, as a function of droplet diameter by creating a histogram with a bin width of 2 μm for droplets with diameters ranging from 2 μm to 10 μm (Fig. 3). Droplets with a diameter larger than 10 μm were grouped together into the final bin. Droplets that were not observed to exhibit the radial configuration were mainly observed to exhibit the bipolar configuration (see ESI Fig. S3[†]). Inspection of Fig. 3 reveals that $98 \pm 1\%$ of the droplets with diameters between 2 and 4 μm exhibit radial ordering. However, as the diameter of the droplets increased, we observed the percentage of droplets with a radial configuration to decrease from $95 \pm 3\%$ to $43 \pm 12\%$ for droplets with diameters of $5 \pm 1\text{ }\mu\text{m}$ and $9 \pm 1\text{ }\mu\text{m}$, respectively. Droplets with diameters greater than 10 μm were insensitive to the presence of endotoxin (less than 5 % of the droplets underwent an ordering transition to a radial configuration). As discussed in detail below, this dependence on droplet size is consistent with our hypothesis that endotoxin-triggered ordering transitions occur in a size regime where the contributions of the elastic and surface anchoring energies to the free energies of the bipolar and radial droplets are nearly balanced. Specifically, the absence of the ordering transition in droplets with sizes larger than 10 μm suggests that, for droplets in this size range, the effects of the surface anchoring energy (which promote the bipolar configuration) cannot be overcome by the interactions of endotoxin with the LC droplets.

As discussed in the Introduction (and detailed below), the size-dependent scaling of elastic and surface anchoring energies leads to the prediction that there should also be a spontaneous transition to a radial configuration *in the limit of small droplet size in the absence of endotoxin*.⁶ As shown in Fig. 3, when 5CB droplets were dispersed in PBS buffer with pH = 7.2, however, we did not observe droplets within the experimentally observable size range (sizes as small as 2 μm) to exhibit a spontaneous transition to a radial configuration in the absence of endotoxin. This result suggests that the size-dependent transition to the radial configuration (in absence of endotoxin) for LC droplets dispersed in PBS at pH 7.2 occurs at a size below 2 μm . When the pH of the buffer was raised to 12.8, however, we did observe LC droplets with diameters less than approximately $3 \pm 1\text{ }\mu\text{m}$ to exhibit the radial configuration and LC droplets with sizes greater than $3 \pm 1\text{ }\mu\text{m}$ to exhibit the bipolar configuration (see ESI Fig. S4[†]). This result is discussed further in the context of a simple thermodynamic model that is presented below.

In light of the influence of LC droplet size on the endotoxin-triggered ordering transitions, we hypothesized that changes in the surface anchoring energy of the 5CB droplets should also lead to changes in the fraction of droplets that undergo endotoxin-induced ordering transitions. In the experiments reported in this paper, we tested this hypothesis by exploiting the results of a recent study that demonstrated that the presence of simple electrolytes in bulk aqueous solution can generate an electrical double layer on the LC-side of the aqueous-LC interface to change the anchoring energy of the LC.¹⁸ Specifically, the electric field associated with the double layer exerts a torque on the LC to promote an alignment of the axis of the molecules with the highest dielectric constant (the long axis in the case of 5CB) parallel to the field. Previously, it has been shown that as the ionic strength or pH of the bulk aqueous solution is increased, the torque changes the surface anchoring energy, leading ultimately to a transition from tangential to homeotropic ordering at the aqueous-5CB

interface.¹⁸ In the experiments reported below, we altered the anchoring energy of the 5CB droplets by changing the ionic strength and pH of the PBS buffer.

First, we investigated the role of the ionic strength of the buffer on the ability of 5CB droplets to undergo an ordering transition in the presence of endotoxin. To initiate our investigation, we dispersed 5CB droplets in increasingly dilute solutions of PBS buffer containing endotoxin, and measured the percentage of the droplets in the radial configuration. Upon dilution, we confirmed that the pH of each solution remained at 7.2. We limited our observations to droplets with diameters between 4 and 6 μm because, as reported in Fig. 3, this size range leads to endotoxin-triggered ordering transitions in PBS at pH 7.2 with an ionic strength of 90 mM. The total ionic strength of each dilution, as well as the ionic strength of each component in the PBS buffer is shown in the ESI (see ESI Table S1[†]). Inspection of Fig. 4 reveals that the percentage of 5CB droplets observed to undergo an endotoxin-triggered ordering transition to a radial configuration decreased with decreasing ionic strength. For example, the fraction of droplets exhibiting the radial configuration was $80 \pm 2\%$ at an ionic strength of 90 mM, and it was measured to decrease to $2 \pm 2\%$ at an ionic strength of 9 mM.

The results shown in Fig. 4 are consistent with the predicted effect of ionic strength on the anchoring energy of the 5CB at the aqueous-LC interface. Specifically, our prior study demonstrated that a decrease in the ionic strength of the aqueous phase increases the Debye screening length of the electrical double layer on the LC-side of an aqueous-LC interface.¹⁸ This increase in Debye screening length weakens the extent to which the double layer promotes the homeotropic orientation. The loss of the endotoxin-induced ordering transition upon decrease in the ionic strength of the buffer is consistent with an increase in strength of the anchoring of LC in an orientation that is tangential to the surface of the LC droplet.

Next, we investigated the effect of the pH of the buffer on the endotoxin-induced ordering transitions. Droplets of 5CB were dispersed in PBS buffer containing 100 pg/mL of endotoxin with pH values of 5.0, 6.0, 7.0, 7.2 and 8.0. Again, we characterized the ordering of LC within droplets with diameters ranging between 4 and 6 μm . In endotoxin solutions with pH values between 7.0 and 8.0, we observed ~95 % of the droplets to undergo an ordering transition to a radial configuration (Fig. 5). However, we observed the fraction of droplets in the radial configuration to decrease with pH below 7.0. For example, in endotoxin solutions with a pH of 6.0 and 5.0, we observed $85 \pm 3\%$ and $52 \pm 1\%$ of the droplets to undergo an ordering transition to a radial configuration, respectively. These results can also be interpreted in terms of the impact of pH on the charging of the aqueous-LC interface, and thus the contribution of an associated electrical double layer to the anchoring energy of the 5CB at the interface. Consistent with this interpretation, Fig. 6A shows that the aqueous-LC interface acquired a less negative potential at the aqueous shear-plane as a function of decreasing pH. Over the range of ionic strengths reported in Fig. 4, however, the change in the zeta-potential was small compared to the uncertainty in the experimental measurement (Fig. 6B). We also comment that the zeta potential characterizes the electrical double layer on the aqueous side of the interface whereas the anchoring energy of the LC is influenced by the electrical double layer on the LC side of the interface.¹⁸ We refer the reader to a past publication that discusses this point in detail.¹⁸

Discussion

The results presented above reveal that the percentage of water-dispersed, micrometer-sized droplets of 5CB that undergo an ordering transition from a bipolar to a radial configuration in the presence of pg/mL concentrations of endotoxin decreases with increasing droplet size. Additionally, decreasing the ionic strength or pH of the aqueous phase also causes a

decrease in the fraction of droplets that undergo the ordering transition in response to pg/mL concentrations of endotoxin; an effect that we associate with changes in the surface anchoring energy of the droplets. Below, we discuss these results in the context of a simple thermodynamic model for the endotoxin-triggered bipolar-to-radial ordering transitions in micrometer-sized LC droplets. We discuss the impact of droplet size, and the ionic strength and pH of the aqueous phase on the various contributions to the free energies of the LC droplets.

We consider the free energy of a LC droplet (F) to be described as

$$F = \int_S f_s dA + \int_V f_e dV + \int_{V_d} f_d dV_d \quad (1)$$

where A is the surface area of a droplet, V is the volume of a droplet, and f_s and f_e are the surface anchoring energy and elastic free energy densities, respectively.¹⁴ We also note that we include a term in equation 1 that describes the self-association of endotoxin with the LC droplet (f_d),¹⁰ where V_d is a volume that encompasses the endotoxin (see below for additional details). In the above expression, the surface anchoring energy density can be approximated using the single-constant Rapini and Papoular form

$$f_s = \frac{1}{2} W \sin^2 \varphi \quad (2)$$

where φ is the angle between the director ($\mathbf{n}$) and the preferred orientation of the LC at the interface (easy axis), and W is surface anchoring energy per unit area.³¹ The elastic free energy density is given by the Frank-Oseen equation

$$f_e = \frac{1}{2} \left[K_{11} (\nabla \cdot \mathbf{n})^2 + K_{22} (\mathbf{n} \cdot \nabla \times \mathbf{n})^2 + K_{33} (\mathbf{n} \times \nabla \times \mathbf{n})^2 - K_{24} \nabla \cdot (\mathbf{n} \times \nabla \times \mathbf{n} + \mathbf{n} (\nabla \cdot \mathbf{n})) \right] \quad (3)$$

where K_{11} , K_{22} , K_{33} and K_{24} are the splay, twist, bend, and saddle-splay elastic constants, respectively.^{15, 17, 32} In the above expression, we have neglected the contribution from the splay-bend elastic constant (K_{13}) because it involves higher order derivatives of $\mathbf{n}$.^{33, 34}

First we consider the energetics of the LC droplets in the absence of endotoxin. Recent theoretical studies predict that K_{24} is important in determining the equilibrium director configuration of LC droplets.^{6, 16, 17} Specifically, the free energy of a droplet in a radial configuration (F_r) can be approximated using equations 1 through 3 as

$$F_r = 8\pi K_{11} R - 4\pi K_{24} R + 2\pi W R^2 \quad (4)$$

The first term is associated with splay deformation within the LC droplet, and the third term is the energetic penalty associated with a homeotropic orientation of the LC at the droplet interface (when the easy axis is tangential).⁶ The second term is associated with saddle-splay deformation of the LC, and favors the radial configuration.^{6, 17} The tendency of K_{24} to lower the elastic free energy penalty associated with the radial configuration gives K_{24} a central role in determining the relative stability of radial and bipolar configurations.^{6, 16, 17}

Adding the contribution of K_{24} to the expression derived by Dubois-Violette and Parodi,¹³ the free energy of a droplet with a bipolar configuration (F_{bp}) can be approximated as

$$F_{bp} = 5\pi K_{11}R - 2\pi K_{24}R \quad (5)$$

where K_{11} and K_{33} are assumed to be approximately equal in magnitude. In equation 5, there is no contribution from the anchoring energy because in aqueous solutions free of adsorbates the preferred orientation of the LC is tangential to the interface ($\phi = 0$).^{5, 6, 10}

The change in free energy upon transforming a LC droplet from a bipolar configuration to a radial configuration ($\Delta F^{bp \rightarrow r}$) can be obtained from equations 4 and 5 as

$$\Delta F^{bp \rightarrow r} = 3\pi K_{11}R - 2\pi K_{24}R + 2\pi WR^2 \quad (6)$$

The model predicts several key behaviors consistent with our experimental observations in the absence of endotoxin. First, when the value of K_{24} exceeds 1.5 times the value of K_{11} ($K_{24} > 1.5K_{11}$), the contribution of the elastic energy to $\Delta F^{bp \rightarrow r}$ favors the radial configuration over the bipolar configuration. Experimental measurements of K_{24} for 5CB suggest it is possible to meet this criterion.^{25, 35-37} For example, through the use of polarized light microscopy, Polak et al. optically determined an apparent value of K_{24} to be 3.1 times the value of K_{11} in systems containing 5CB confined to capillary tubes.²⁵ Although this value exceeds the so-called Ericksen limit ($K_{24} = 1.5K_{11}$),³⁸ we note that a value of $K_{24} = 1.53K_{11}$ stabilizes the radial configuration. We also note that equation 6 is approximate (see above) and should not be expected to quantitatively predict the behaviour of the system.

When the contribution of the elastic energy to $\Delta F^{bp \rightarrow r}$ favors the radial configuration, adoption of the radial configuration by the LC droplet requires that it overcome the anchoring energy (equation 6). The model predicts, therefore, that the contributions of the elastic and anchoring energy to $\Delta F^{bp \rightarrow r}$ will balance at a critical radius defined by $R_c = (K_{24} - 3/2K_{11})/W$. Below R_c , the radial configuration possesses a lower free energy whereas above R_c , the bipolar configuration corresponds to the lower free energy configuration. This prediction of the model is consistent with our previous observation of a bipolar-to-radial ordering transition within polymer-encapsulated 5CB droplets when the diameters of the droplets were below approximately 800 nm.⁶ Using this value of the critical radius and the limiting value of $K_{24}(1.53K_{11})$, we calculate $W \approx 5.0 \times 10^{-7} \text{ J/m}^2$ for polymer-encapsulated 5CB droplets. We note that this value of W corresponds to relatively weak anchoring conditions at the surfaces of the droplets.^{14, 39, 40} In the present system, our results indicate that R_c falls below 1 μm for 5CB droplets dispersed in PBS buffer at pH = 7.2 (Fig. 3). However, when the pH of the buffer was raised to 12.8, a value of R_c of approximately 1.5 μm was observed (see ESI Fig. S4[†]). Similar to the polymer-coated 5CB droplets, this value of R_c results in an estimate of $W \approx 1.3 \times 10^{-7} \text{ J/m}^2$.⁶

To describe the influence of endotoxin on the relative stability of the radial and bipolar configurations of the LC droplets, we modified equation 6 to

$$\Delta F^{bp \rightarrow r} = 3\pi K_{11}R - 2\pi K_{24}R + 2\pi WR^2 + \Delta F_{endotoxin} \quad (7)$$

Where $\Delta F_{endotoxin}$ describes the relative influence of endotoxin on the free energies of the radial and bipolar droplets (see equation 1). While very simple, this model yields several significant conclusions. First, as the size of the 5CB droplets increases above a threshold value of R , the balance between the elastic and anchoring energies will be lost because the contribution of the anchoring energy (proportional to R^2) will become dominant over the contribution of the elastic energy (proportional to R). Under these conditions, changes in the free energy due to the interactions of the LC droplets with endotoxin ($\Delta F_{endotoxin}$) will no

longer be sufficiently large to drive the ordering transition. Our observations presented in Fig. 3 are consistent with this prediction, as droplets above a diameter of 10 μm did not undergo an ordering transition from a bipolar to a radial configuration in the presence of 100 pg/mL endotoxin (threshold radius of $\sim 5 \mu\text{m}$). Second, equation 6 can be combined with our experimental observations to estimate the magnitude of $\Delta F_{\text{endotoxin}}$. At the threshold radius defined above (5 μm), the magnitude of $\Delta F_{\text{endotoxin}}$ should be approximately equal to the combined contributions of the elastic and anchoring energies in equation 6. Insertion of this threshold radius into equation 6 along with $K_{24} = 1.53K_{11}$ and $W = 4.0 \times 10^{-7} \text{ J/m}^2$ (corresponding to $R_c = 500 \text{ nm}$) results in an estimate of $|\Delta F_{\text{endotoxin}}|$ of approximately 10^{-17} J .

The above-described estimate of $|\Delta F_{\text{endotoxin}}|$ is interesting to consider in light of past observations of accumulation of endotoxin at the center of LC droplets in the radial configuration.¹⁰ Indeed, past studies with colloidal particles and blue phases of LCs have reached the conclusion that it is possible to stabilize blue phases via a “defect core replacement” mechanism.⁴¹ The nanoparticles stabilize blue phases by displacing LC from the cores of topological defects, effectively reducing the large energetic penalty associated with the defects.⁴¹ To determine if such a mechanism might give rise to an effect of order 10^{-17} J with endotoxin, we estimated the energy of a point defect as $E_{\text{core}} \sim 4\pi/3r_c^3\epsilon_c$, where r_c is the radius of the core of the defect and ϵ_c is the free energy of melting a nematic phase to an isotropic phase.^{42, 43} The size of the core can be calculated from the competing effects of the elastic energy density and melting energy density as $r_c \sim (K/\epsilon_c)^{0.5}$, resulting in $E_{\text{core}} \sim 4\pi/3\epsilon_c^{-0.5} K^{1.5}$.⁴³ By approximating ϵ_c to be the same magnitude as the enthalpy of the nematic to isotropic transition ($\sim 10^6 \text{ J/m}^3$),⁴⁴ E_{core} is calculated to be on the order of 10^{-19} J , which is two orders of magnitude lower than the above-described experimental estimate of $|\Delta F_{\text{endotoxin}}|$. This result suggests that the influence of endotoxin, if through localization at defects, is not simply via a “core replacement” mechanism, as has been proposed for the colloidal stabilization of blue phases of LCs over broad temperature ranges.^{41, 45-47}

To provide further insight into the possible origin of the above-described experimental estimate of $|\Delta F_{\text{endotoxin}}|$, we consider two observations from a previous study.¹⁰ First, ordering transitions in LC droplets are triggered by pg/mL concentrations of endotoxin but not pg/mL concentrations of 1,2-dilauroyl-*sn*-glycero-3-phosphatidylcholine (DLPC), 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), or synthetic surfactants such as sodium dodecylsulfate (SDS).¹⁰ The dependence of the ordering transitions on the lipid architecture of endotoxin leads us to the hypothesis that self-assembly of endotoxin with the LC droplet drives the ordering transitions. Consistent with this hypothesis, we calculate the magnitude of the standard free energy of the self-association of 10^3 endotoxin molecules to be on the order of 10^{-17} J .^{48, 49} Second, although occupation of the core of defects by endotoxin is insufficient to account for the magnitude of $|\Delta F_{\text{endotoxin}}|$, as noted above, accumulation of endotoxin at the location of the central defect in the LC droplets has been observed.¹⁰ The magnitude of $\Delta F_{\text{endotoxin}}$, when combined with this past imaging, is consistent with the hypothesis that endotoxin lowers the energy of the radial configuration relative to the bipolar configuration through formation of an organized lipid assembly located at the center of the radial configuration. We note also, however, that the nanoscopic organization of the endotoxin at the center of the LC droplet is currently unknown, and that additional investigations are needed to validate this hypothesis.

A recent theoretical study predicts that the flexoelectricity of nematic LCs can induce an electrostatic potential at topological defects contained within LC droplets.⁵⁰ The presence of a positive electrostatic potential near the core of topological defects might provide some of

the driving force for the localization of negatively charged endotoxin molecules at these defects.¹⁰ The testing of this hypothesis is the focus of an ongoing investigation.

Finally, we note that the predictions of the above-described model for endotoxin-induced changes to the free energy of LC droplets are dependent on the LC anchoring energy. An increase in anchoring energy will decrease R_c , and thus for a given size of LC droplets, the fraction of droplets that undergo an ordering transition to the radial configuration in the presence of pg/mL concentrations of endotoxin is predicted to decrease. Our observations (Figs. 4 and 5) are consistent with this prediction, as decreases in both the ionic strength and pH of PBS buffer led to a decreased fraction of 5CB droplets that undergo an ordering transition from a bipolar to a radial configuration. As discussed in detail above, decreasing the ionic strength and pH of PBS buffer has been shown previously to increase the strength of anchoring of LC at the aqueous-LC interface in an orientation that is tangential to the interface due to a reduction in electrical double layer forces on the LC-side of the aqueous-5CB interface.¹⁸ Overall, the results in Figs. 4 and 5 are consistent with the prediction that the stronger anchoring of the LC in an orientation tangent to the aqueous-LC interface disrupts the near balance of elastic and anchoring energies necessary to observe an endotoxin-triggered ordering transition.

Conclusions

In summary, the experiments reported in this paper advance our understanding of the origins of endotoxin-induced ordering transitions in micrometer-sized LC droplets that are triggered by pg/mL concentrations of endotoxin. Several key experimental results reported in this paper support the proposition that the ordering transitions are observed under conditions where the contributions of the bulk elastic and surface anchoring energies to free energy of LC droplets in the bipolar and radial configurations are close to degenerate. Specifically, we observe the ordering transitions induced by endotoxin to be strongly dependent on the size of the LC droplets and solution conditions that are expected to influence the surface anchoring energy of the LC at the aqueous interface (pH and ionic strength). These observations are consistent with a simple (order of magnitude) thermodynamic model that describes elastic and surface anchoring contributions to the LC droplet configurations. This model also yields an estimate of the endotoxin-induced change in the droplet free energy (approximately 10^{-17} J) required to cause an ordering transition. The magnitude of this term is much larger than a core defect energy but similar to the change in free energy associated with self-assembly of $\sim 10^3$ endotoxin molecules with each LC droplet. When combined with prior reports of localization of endotoxin at the centers of LC droplets, these results are consistent with the proposition that self-assembly of endotoxin at the centers of LC droplets (of a narrow size range) can provide the driving force for the ordering transitions. In a broader context, the insights reported in this paper advance the design of LC droplet systems as stimuli-responsive soft materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Schematic illustrations of the orientational ordering of LC within a droplet in a (A) bipolar configuration and the (B) radial configuration. In a bipolar configuration LC is oriented parallel to the interface of the droplet and two diametrically opposite surface point defects (called boojums) are present at the poles of the droplet, whereas in the radial configuration LC is oriented perpendicular to the interface of the droplet and a defect is present at the core of the droplet.

Fig. 2.

Influence of the size of 5CB droplets dispersed in PBS buffer (pH = 7.2) on the response of the droplets to the presence of 100 pg/mL endotoxin in solution. (A-D) In the absence of endotoxin, droplets with diameters of $6.5 \pm 0.5 \mu\text{m}$ (A and B) and $10.5 \pm 0.5 \mu\text{m}$ (C and D) exhibit a bipolar configuration. (E-F) Upon addition of endotoxin, droplets with diameters of $6.5 \pm 0.5 \mu\text{m}$ (E and F) undergo a bipolar-to-radial ordering transition, whereas a majority of droplets with diameters of $10.5 \pm 0.5 \mu\text{m}$ (G and H) remain in the bipolar configuration. BF indicates a bright field micrograph and PM indicates a polarized light micrograph (crossed polars). The single headed arrows indicate the position of defects in the droplets and the double headed arrows indicated the orientation of the crossed polars. Scale bars, 5 μm .

Fig. 3.

Histogram of the size-dependent response of 5CB droplets to the presence of 100 pg/mL endotoxin in PBS buffer, pH = 7.2. The percentage of 5CB droplets that exhibited a radial configuration both in the presence (solid bar) and absence (open bar) of 100 pg/mL endotoxin is plotted for each droplet diameter range. No 5CB droplets were observed to exhibit a radial configuration in the absence of 100 pg/mL endotoxin above a diameter of 6 μm .

Fig. 4. Percentage of 5CB droplets that exhibited a radial configuration in 40 μL of PBS buffer containing 10 pg/mL of endotoxin, plotted as a function of the total ionic strength of the PBS buffer solution. The droplets analyzed had diameters of 4 to 6 μm . Note that we demonstrated previously that LC droplets in PBS at pH 7.2 (ionic strength of 90mM) exhibit a radial configuration in the presence of 10 pg/mL of endotoxin.¹⁰

Fig. 5. Percentage of 5CB droplets that exhibited a radial configuration in 40 μL of PBS buffer containing either ($\blacklozenge$) 100 $\mu\text{g/mL}$ of endotoxin or ($\blacksquare$) no endotoxin, plotted as a function of the pH of the PBS buffer solution. The droplets analyzed had diameters of 4 to 6 μm .

Fig. 6. (A) Zeta potential (ζ) of 5CB droplets in PBS buffer with an ionic strength of 90 mM plotted as a function of the pH of the buffer. (B) Zeta potential of 5CB droplets in PBS buffer with pH = 7.2 plotted as a function of the percentage of the total ionic strength of the PBS buffer.