Positional Order in the Columnar Phase of Lyotropic Chromonic Liquid Crystals Mediated by Ionic Additives

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ABSTRACT: Positional order in the lyotropic chromionic liquid crystals (LCLCs) is investigated in the supramolecular assembly of benzene 1,3,5-tricarboxamide (BTA) derivatives with the glucono-delta-lactone (GdL) acid additive by high-resolution synchrotron radiation small-angle X-ray scattering. The formation of positionally ordered hexagonal phase is found to profoundly depend on the concentrations of BTA derivatives, $c_{BTA}$, and GdL additives, $c_{addtive}$, giving rise to unusual behavior distinctive from conventional lyotropic liquid crystals (LCs) with covalent bonds and fixed length. The hexagonal phase is observed to coexist with another phase in certain range of $c_{addtive}/c_{BTA}$. Intriguingly, the lattice spacing $R$ of the hexagonal phase remains almost constant by varying $c_{addtive}$, but changes with $c_{BTA}$. The above observations are attributed to unique sensitivities of the LCLC properties, such as the contour length and flexibility of individual cylinder assemblies and phase coexistence, to additives in the solutions. Our study reveals the complexity in positional ordering in the LCLCs which not only relates to the underlying principles of hierarchical reversible self-assembly but also attracts fundamental interests in LCs.

INTRODUCTION
The interest in lyotropic liquid crystals (LLCs) composed by amphiphilic molecules in water solutions has grown in the last decades because of their potential applications. One relative new and interesting class of LLC is based on an aromatic type of chromonic molecules with a relatively rigid disc-like core and ionic/polar groups at the periphery, which is distinct from the conventional LLC of flexible, rod-like aliphatic amphiphiles such as surfactants and biological lipids. This class of LLC embraces a range of drugs, dyes, nucleic acids, antibiotics, and synthetic benzene 1,3,5-tricarboxamide (BTA) derivatives, finding potential applications in water-solution processable organic electronics and devices for the detection of specific antibodies in medical diagnosis. Their supramolecular aggregation is often driven by the tendency of aromatic rings to stack face-to-face, resulting in polydispersive stacks or cylinders rather than micelles. Within certain concentration range, the supramolecular assemblies further form the LC phase with long-range orientational even positional order. The liquid crystalline properties of such chromonic systems, the so-called lyotropic chromonic liquid crystals (LCLCs), are essential to their application. The LCLCs are distinct from conventional LLCs of covalently bound units with fixed length such as DNA molecules. Because of the relatively weak noncovalent interaction, the average length of the assemblies varies with concentration, temperature, ionic strength, and additives. In addition, the assemblies are flexible similar to worm-like polymers as suggested by studies on elasticity and rheology. These unique properties of the LCLCs as reversible assemblies together with the peripheric ionizable groups make the LCLCs very sensitive to solution environments. Ionizable additives such as salts and acids profoundly affect the self-assembly of LCLC through modulating electrostatics within and between the assemblies.

Experimental studies have revealed dramatic and versatile influences by ionizable additives on the rheology, viscoelastic properties, and phase diagrams of the LCLCs. Some of these results can be understood through two tendencies in terms of the individual assembly structure because of the screening of electrostatic repulsion by ionic additives. First, the screening of electrostatic repulsion between the molecules results in elongation of assemblies or increment of their contour length. Salt-triggered elongation of assemblies has been observed experimentally and supported by thermodynamic models. Similar phenomenon is also discovered for protonation of carboxylate terminals around BTA derivatives by adding acids. Another potential result of electrostatic screening is the increased flexibility of the assemblies or shortening of their persistence length $P$. According to the Manning model, charged segments of a flexible charged rod experience Coulomb repulsions, which produces stretching force and enhances the bending rigidity of the rod. When the repulsions are screened by ionic contents, the rod becomes more flexible. The decrease of persistence

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length $P$ by ionic additives is commonly observed in water solutions of worm-like surfactant micelles, flexible polyelectrolytes, and biomacromolecules. The modification in contour length and persistence length leads to substantial changes in the ability of assemblies to form LCs. Salt-induced shift in the boundary between isotropic phases and LC phases has been intensively investigated for the LCLCs.

Although the mechanisms underlying the formation of the LCLCs have been widely explored in pure materials and in the presence of additives, the long-range positional order remains largely unexplored in LCLCs with additives. The majority relating studies are focused on the correlation length or stability of the hexagonal phase instead of interassembly separation in the hexagonal phase. In contrast, there is substantial amount of knowledge accumulated on positional order in LLCs of covalent counterpart such as DNA and other biomacromolecules with fixed shape and length. For LLCs, the positional order in the hexagonal phase is controlled by complicated interassembly interactions including excludes volume effects, electrostatic and hydration forces. These forces profoundly respond to the chemical environment in the solution through various factors such as the contour length and persistence length of the assemblies, ion partitioning, and so on. In particular, electrostatics for semiflexible polyelectrolytes is revealed to be much more complicated than rigid charged rods. The coupling between electrostatics and thermal fluctuations or undulation of semiflexible polyelectrolytes has been demonstrated in LLC of DNAs. As mentioned previously, the properties of assemblies by chromonic molecules are sensitive to additives so that the positional order in the LCLCs with ionizable additives will be quantitatively different from covalent bond counterparts.

Based on the hexagonal phase formed by the supramolecular assembly of ionic BTA derivatives in aqueous solution of glucono-delta-lactone (GdL), we investigate how the positional order, in particular, the lattice spacing $R$ of the hexagonal phase, varies with both component concentrations in the LCLC system. The slow hydrolysis process and release of proton of GdL in water can facilitate the formation of assemblies by protonation of peripheral COO$^-$ groups around the BTA derivatives. The positional order in the LCLC system is investigated by synchrotron radiation small-angle X-ray scattering (SAXS) at various concentrations of BTA and GdL. Hexagonal phases are observed in certain range of GdL concentration, which however has little influence on the lattice spacing $R$. $R$ mainly depends on the concentration of BTA, in a nontrivial way different from the pure hexagonal phase or coexisting phases commonly observed for LLCs. These phenomena have not been observed for LLC of polyelectrolytes but may be understood through the properties of individual assemblies as well as phase diagram that are unique for the LCLCs.

### RESULTS AND DISCUSSION

BTA derivatives with rigid arms and COO$^-$ end groups in Figure 1 are synthesized and characterized (see part 1, Supporting Information). BTA derivatives self-assemble into supramolecular columns in solution and in solids through intermolecular $\pi-\pi$ stacking and H-bonding. BTA derivatives with very similar structure have been reported to form supramolecular chromophores by adding GdL. Here, this particular BTA derivative is used to study the long-range positional order in the LCLCs with additives. The solution samples are prepared by dissolving BTA derivatives and GdL in de-ionized water. The pH of the mixture gradually decreases with time from about 7 to about 4 because of the hydrolysis of GdL and subsequent release of protons (see Figure S6 in Supporting Information). Upon acidification, the peripheral COO$^-$ groups are protonated, which reduce intermolecular electrostatic repulsion as well as molecular solubility to enable the supramolecular assembly. The mixture solutions are further aged for about 10 weeks to achieve equilibrium of assembly. The turbidity of aged sample shown in Figure 1b comes from supramolecular assemblies dispersed in the solution, which may precipitate out upon centrifugation as...
shown in Figure S7 in the Supporting Information. Transmission electron microscopy (TEM) on the negative stained sample in Figure 1c demonstrates the formation of supramolecular fibrils with diameters about 5 nm and lengths exceeding 1 μm. POM pictures in Figure 1d suggest several phases coexisting in the solutions as observed in many LCLCs.\(^{14,19,27}\)

Acid or proton-promoted assembly is further investigated by varying the concentration of GdL additive \(c_{\text{additive}}\) at fixed concentration of BTA derivatives. Photos of aged samples in Figure 2a show the transformation from almost transparent to turbid solutions as \(c_{\text{additive}}\) increases. The increased turbidity of the solution is because of the formation of more assemblies. Similar phenomenon has been observed for pH-dependent formation of supramolecular chromophores by adding GdL into the solution of BTA.\(^{27}\) At low \(c_{\text{additive}}\) Protonation of the COO\(^{−}\) end groups is rather limited and so is the degree of supramolecular assembly. The solutions appear quite translucent for samples with \(c_{\text{additive}}\) lower than 13 mmol/L even after aging. As \(c_{\text{additive}}\) increases, more COO\(^{−}\) groups are protonated, and more supramolecular assemblies are formed. The promotion of supramolecular assembly by additives is also manifested by the length of assemblies. TEM suggests the presence of segments shorter than 200 nm at a GdL concentration of 13 mmol/L in Figure 2b while no such segments are observed in solutions of 17 mmol/L GdL in Figure 2c. This observation is in line with the enhanced cooperativity and elongation in the supramolecular assembly with ionic strength reported for other BTA derivatives\(^{36}\) and many LCLCs.\(^{18,20,27}\)

As the length and concentration of supramolecular assemblies increase, the assemblies spontaneously align parallel to each other and pack into bundles. The higher order between assemblies is studied in solution by SAXS. Representative SAXS in Figure 3a shows a primary sharp Bragg peak accompanied by weaker higher order peaks, indicating a long-range positional order. Quantitative structural information can be achieved by fitting the SAXS curve with multiple Lorentzian curves after subtracting a diffuse background peak as demonstrated in Figure 3a. The ratios of the peak positions relative to the primary first order peak follow the sequence of 1:\(\sqrt{3}/2\), suggesting the formation of hexagonal phase, which is very common for the LCLCs.\(^{14,15,19,27}\) The transverse two-dimensional electron density distribution is derived from SAXS data by Fourier transform and shown in Figure 3b. The red color corresponds to higher electron density, namely the axial center of supramolecular assemblies. The blue color corresponds to low electron density of continuous water background. Yellow corresponds to the arms with intermediate electron density. The diameters of supramolecular assemblies can be estimated to be about 5 nm, in agreement with TEM results. The diameters are much larger than those of the BTA derivatives around 3 nm, suggesting multiple molecules per stacking layer.\(^{3,37}\) Normally, the number of molecules per layer can be derived from the Luzzatti log/log plot,\(^{3}\) which is however not feasible here because of phase-coexistence as discussed later, so that only very schematic models of molecular assembly are proposed in Figure S8. In the proposed multiple-molecule stacks, a few molecules are linked together into layers which pile up and form the cylindric assemblies.

Interesting response of the positional order to the extent of acidification or protonation is displayed in the color contour plot of SAXS in Figure 4, which is measured on solutions with varying concentrations of GdL \(c_{\text{additive}}\) at fixed concentrations of BTA \(c_{\text{BTA}} = 40\) and 85 mmol/L. The contour plot is a false color plot of SAXS intensities as a function of \(Q\) (X axis) and \(c_{\text{additive}}/c_{\text{BTA}}\) (Y axis). The sharp red stripe-like features at smaller \(Q\) depict the signature first-order Bragg peak of the hexagonal phase. At \(c_{\text{BTA}} = 40\) mmol/L in Figure 4a, the red stripe is located around \(Q = 0.6\) nm\(^{−1}\). Surprisingly, the stripe only slightly shifts from 0.61 to 0.58 nm\(^{−1}\) as \(c_{\text{additive}}\) increases from 6.5 to 17 mmol/L (\(c_{\text{additive}}/c_{\text{BTA}}\) from about 0.16 to 0.42 in the plot), corresponding to an expansion of lattice \(R = 4z/\sqrt{3}/Q\) from 11.9 to 12.5 nm. At higher \(c_{\text{BTA}} = 85\) mmol/L in Figure 4b, the sharp stripe is located at larger \(Q\) between 0.68 and 0.71 nm\(^{−1}\) in a rather wide range of \(c_{\text{additive}}\).
Figure 4. Dependence of positional order on \( c_{\text{additive}} \) in the LCLC hexagonal phase. SAXS color contour plot at various mole ratios of GdL additive relative to BTA derivatives in solutions with BTA concentration \( c_{\text{BTA}} \), fixed at (a) 40 and (b) 85 mmol/L, respectively. Color scale on the right represents the scattering intensity normalized to the most intense feature. In most cases, the sharp first order Bragg peak is the most intense feature and is manifested as a red stripe in the contour plot in the lower Q region. Exception exists in (b) for \( c_{\text{additive}}/c_{\text{BTA}} \) lower than 0.15 or higher than 0.35, where the broad diffusive feature is the most intense and shown as a red bump at higher Q values.

Figure 5. Dependence of R, the lattice spacing in the hexagonal phase, on \( c_{\text{BTA}} \). (a) SAXS characterization of the LCLCs at a fixed mole ratio of GdL relative to BTA derivatives at 0.3:1. The numbers above each curve denote \( c_{\text{BTA}} \). Inserted is the log–log plot of \( R = 4\pi/(\sqrt{3}Q) \) derived from Q of the first order peak vs \( c_{\text{BTA}} \). The dotted line is the apparent linear fit of the log–log plot. (b,c) Schematic illustration of the configurational entropy \( \Delta F_{\text{config}} \) of semiflexible assemblies because of axial undulation \( d \) and its competition with interassembly electrostatic interaction \( \Delta F_{\text{elec}} \) at various \( c_{\text{BTA}} \). The transverse cross-section of the hexagonal phase is indicated by a yellow hexagon with side length of R. Individual assembly subjected to thermal undulation is represented by a worm-like chain confined in a tube with diameter \( d \).

larger than 0.35 for \( c_{\text{BTA}} = 85 \) mmol/L in Figure 4b. Similar suppression of positional order by salts has been observed for LCLC of sunset yellow, a widely studied chromonic material, attributed to the increased flexibility or axial undulation \( d \) of the assemblies by salts. In Figure 4b, the positional order vanishes at larger \( c_{\text{additive}}/c_{\text{BTA}} \) because of reduced surface charge density, \( d \) may grow too big to stabilize the hexagonal phase, and the positional order vanishes at larger \( c_{\text{additive}}/c_{\text{BTA}} \) in Figure 4b. In a wide range of BTA concentrations, the LCLC hexagonal phases are formed in a certain range of \( c_{\text{additive}}/c_{\text{BTA}} \) as summarized in the schematic phase diagram in Figure S9. For smaller \( c_{\text{additive}}/c_{\text{BTA}} \), the amount and length of assemblies are not sufficient to form hexagonal phases but form less-ordered phases as observed in many other LCLCs and manifested by the broad features in Figure 4. For larger \( c_{\text{additive}}/c_{\text{BTA}} \), the positional order will be suppressed possibly because of increased flexibility as discussed later so that less-ordered phases dominate the LCLC system. Because R is not sensitive to \( c_{\text{additive}} \) the hexagonal phase can be studied by varying \( c_{\text{BTA}} \) at fixed \( c_{\text{additive}}/c_{\text{BTA}} = 0.3 \) as summarized in Figure 5. As \( c_{\text{BTA}} \) increases, the first order Bragg peak apparently grows and shifts toward larger \( Q \), suggesting more and denser packing of the hexagonal phases in the LCLCs. Concentration dependence of lattice spacing \( R \) is common in the pure hexagonal phase, which follows the so-called 2D swelling rule. In the inset of Figure 5, the lattice spacing is plotted as a function of \( c_{\text{BTA}} \) in the log–log plot, which surprisingly gives a linear relationship with the slope about −0.24 instead of −1/2 as expected for 2D swelling. The apparent deviation might be understood through phase coexistence in the solution here while 2D swelling only applies for pure phases. On the other hand, although the coexisting phases normally do not change with concentrations, the presence of additives introduces a new degree of thermodynamic freedom and changes the above requirement of phase coexistence. Therefore, not only the hexagonal phases of the LCLC but also the coexisting phases lacking the positional order get compressed as \( c_{\text{BTA}} \) increases as seen from the broad diffusive features in Figure 4.
Figures 4 and 5 together suggest an unusual phase diagram of the LCLCs with additives that has not been reported in the lyotropic hexagonal phase of rigid rods or covalent counterparts with fixed length. The first unusual behavior is that the lattice spacing \( R \) remains nearly constant at various \( \text{c}_{\text{additive}} \) in Figure 4. The observation contradicts the intuition that the protonation of \( \text{COO}^- \) groups by additives should reduce the electrostatic repulsion and hence the spacing \( R \). The second unusual behavior is the dependence of \( R \) on the concentration of BTA derivatives \( \text{c}_{\text{BTA}} \) in phase coexistence in Figure 5, which neither follows the 2D swelling rule for the pure hexagonal phase nor the thermodynamics for phase coexistence. The latter requires that the structures of coexisting phases shall not vary with concentrations. Normally, unusual behaviors are attributed to the uniqueness of the LCLCs from aspects of individual assembly properties such as the contour length and persistence length, \( R_p \), \( R_{\text{persistence length}} \), which are essential to the formation of positional order. In particular, the persistence length \( P \) and associated axial undulation \( d \) of semiflexible polyelectrolytes have been revealed to be critical to quantitatively understand the positional order in their lyotropic hexagonal phase \( \approx \) as well as the melt of hexagonal phase at high salt concentration \( R_\text{salt} \).

The theoretical foundation of the fluctuation and interaction of semiflexible polyelectrolytes in the hexagonal phase was laid by Odijk \( \approx \) and briefly described below. In the hexagonal phase, the worm-like semiflexible chain is effectively confined in a cylindrical tube of diameter \( d \), causing some lose in configurational entropy \( \Delta F_{\text{conf}} \approx k_B T/(d^2P)^{1/3} \) associated with axial undulation of the chain as schematically illustrated in Figure 5b,c. Meanwhile, the electrostatic repulsion \( \Delta F_{\text{el}} \) between semiflexible chains in a hexagonal phase is enhanced by the undulation through a factor \( \exp(\kappa^2d^2/2) \), where \( \kappa^{-1} \) is the screening length. The undulation \( d \) is determined by the competition between \( \Delta F_{\text{conf}} \) and \( \Delta F_{\text{el}} \), which in turn depends on lattice separation \( R \), ionic strength, surface charge, and persistence length \( P \). We propose that the small change in \( R \) with \( \text{c}_{\text{additive}} \) can be understood by the above scenario as illustrated in Figure S10 of the Supporting Information. As \( \text{c}_{\text{additive}} \) increases, the reduced surface charge density and screening effects shorten the persistence length \( P \) of individual assemblies \( R_p = \text{constant} \). The assemblies then become more flexible and can tolerate larger undulation, which enhance the electrostatic repulsion between them. As \( d \) is positively correlated to \( R_\text{salt} \), so that larger \( R \) is more favorable by \( \Delta F_{\text{conf}} \). The above effects maintain \( R \) nearly constants despite the intuition that higher \( \text{c}_{\text{additive}} \) may reduce electrostatic repulsion. On the other hand, if \( \text{c}_{\text{additive}} \) is high enough, the assemblies become so flexible that \( d \) is too large to stabilize the positional order, and the hexagonal phase vanishes above \( \text{c}_{\text{additive}}/c_{\text{BTA}} \approx 0.35 \) in Figure 4b.

The above interpretation is in line with the effects of additives on the flexibility of chromonic assemblies, which has been inferred from measurements on the elastic constants of the LCLCs using a magnetic Frederiks transition technique \( \approx \). Similar mechanism is also considered for covalent counterparts and individual molecules, such as DNA. In both cases, such effects of additives on the flexibility will impose important influence on the long-range positional order. Undulation-enhanced electrostatics in the positionally ordered LC phase is quite established for polyelectrolytes and biomacromolecules including DNAs. Similar mechanism has also been proposed to understand the phase diagram of LCLC with additives. For flexible assemblies, the stability of LC is determined by \( P \), which not only explains the shift in the isotropic–nematic phase transition but also the melting of hexagonal phase of SSY LCLC at high concentration of NaCl \( \approx \) Although we cannot provide direct experimental evidence on the flexibility because of lack of access to the special equipment required by the new magnetic technique, the interpretation is in the same scenario of the intensively studied case of SSY.

Our results also suggest the influence of additives on the phase diagram of the LCLCs, more specific, on phase coexistence. Phase coexistence is common in LCLCs with or without additives \( \approx \), which consists of a low-density disordered phase and a denser ordered phase. In a pure system without additives, the structures of the coexisting phases shall not change with concentration but only the relative amount of contents does. The additives relax the above constraints on phase coexistence so that the lattice spacing \( R \) of the hexagonal phase evolves accordingly with the solution environments.

Then, the change of \( R \) in Figure 5a can also be qualitatively understood by making analogy to the competition between orientational entropy and packing entropy for rigid hard rods \( \approx \). Although the orientational entropy dominates at low concentrations, packing entropy dominates in concentrated solutions of rods. Their competitions drive LC phase transitions \( \approx \). For semiflexible chains, the orientational entropy is replaced by \( \Delta F_{\text{conf}} \) and the packing entropy by \( \Delta F_{\text{el}} \). At low molecular concentrations, \( \Delta F_{\text{conf}} \approx (d^2P)^{-1/3} \) dominates, which favors larger axial undulation \( d \) and \( R \) as schematically illustrated in Figure 5b. At higher molecular concentration, \( \Delta F_{\text{conf}} \approx \exp(\kappa^2d^2/2) \) becomes more important so that smaller \( d \) and \( R \) are more favorable as illustrated in Figure 5c.

**CONCLUSIONS**

The long-range positional order in the LCLCs of BTA derivatives with the additive described in this work is highly nontrivial and unique even for LLCs. We proposed that such phenomena originate from unique properties of the LCLCs distinct from conventional LLC and covalent bound counterparts with the fixed contour length. In particular, the sensitivity of individual assemblies’ properties, such as contour length and flexibility, to the concentration of the additives in the LCLCs may be critical to understand the observed phase diagram. Furthermore, the additives introduce an additional degree of freedom and greatly change the phase coexisting condition. In LCLCs with ionic additives, many important parameters such as axial undulation, ionic strength, and lattice spacing are correlated and depend on each other in the emergence of long-range positional order, which is in line with complicated electrostatics well established for polyelectrolytes and biomacromolecules. Our results suggest that the quantitative behavior of long-range positional order in the LCLCs is even more intriguing and complicated, which merits further and deep investigation. Further investigation into other factors such as temperature effects will be carried out in the future. Overall, the LCLC exhibits a very interesting and unusual phase diagram, which coincides with emerging topics such as reversible assembly and complex electrostatics between charged soft matters.

**EXPERIMENTAL SECTION**

The materials, synthesis, and characterization section can be found in part 1 Supporting Information. A solution sample is
prepared by dissolving the molecules in de-ionized water. SAXS measurements were carried out at beamline 16B1 in the Shanghai Synchrotron Radiation Facility. The X-ray wavelength $\lambda$ was 0.124 nm, and a Mar165 CCD detector was employed to collect 2D SAXS patterns with sample-to-detector distances about 2025 mm. The sample cell with aqueous solutions sealed between two Kapton membranes were fixed on the sample holder during the measurements. Fit2D software from the European Synchrotron Radiation Facility was used to analyze scattering patterns in terms of the scattering vector $Q$ = $4\pi \sin \theta/\lambda$, with $2\theta$ as the scattering angle. Method of reconstruction of electron density profile from X-ray scattering data for the hexagonal phases (2D periodic systems) can be found in ref 44.

## ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00229. Materials and synthesis, pH of sample, photos of sample before and after centrifugation, schematics of molecular assembly model, schematic phase diagram of the LCLC, and schematics of the flexibility of assembly chain (PDF)

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Notes
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