Video Article

Methods for the Self-integration of Megamolecular Biopolymers on the Drying Air-LC Interface

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Abstract

Living organisms that use water are always prone to drying in the environment. Their activities are driven by biopolymer-based micro- and macro-structures, as seen in the cases of moving water in vascular bundles and moisturizing water in skin layers. In this study, we developed a method for assessing the effect of aqueous liquid crystalline (LC) solutions composed of biopolymers on drying. As LC biopolymers have megamolecular weight, we chose to study polysaccharides, cytoskeletal proteins, and DNA. The observation of biopolymer solutions during drying under polarized light reveals milliscale self-integration starting from the unstable air-LC interface. The dynamics of the aqueous LC biopolymer solutions can be monitored by evaporating water from a one-side-open cell. By analyzing the images taken using cross-polarized light, it is possible to recognize the spatio-temporal changes in the orientational order parameter. This method can be useful for the characterization of not only artificial materials in various fields, but also natural living tissues. We believe that it will provide an evaluation method for soft materials in the biomedical and environmental fields.

Video Link

The video component of this article can be found at https://www.jove.com/video/55274/

Introduction

By focusing on the rigid, rod-shaped structures of biopolymers, dynamic soft materials have been used for various applications, including polysaccharide biofilm matrices[1], "active gels" composed of cytoskeletal proteins[2], and "DNA origami" of desired shapes[3]. To clarify the structural properties, many strategies have been explored, such as transmission electron microscopy, scanning electron microscopy, atomic force microscopy, and confocal fluorescence microscopy. However, because these methods are mostly undertaken in a dried or static state, it is difficult to explain the dynamic behaviors in macroscopic scales, as seen in actual living systems. Recently, we successfully observed the dynamic behavior of biopolymers on the aqueous air-LC interface through polarized light[4]. During the visualization of the oriented structure while drying the biopolymer solution, the temporal changes indicated self-integration of biopolymers on the unstable air-LC interface.

Here, we describe a protocol for the drying of LC biopolymer solutions at the air-LC interface using polarized instruments. As opposed to other analyses of the LC phase that do not consider drying[5,6], the LC dynamics during the drying process were investigated here by evaluating the orientational order parameter in the lateral view of the fluid phase in a one-side-open cell. The combination of the cell evaporation and the use of polarized instruments allowed for macroscopic monitoring with a controlled evaporation direction. In addition, it was possible to validate the drying records by focusing on the crystalline structures of the adsorbed microdomains, which were affected by molecular weight, concentration, *etc.* To demonstrate the effectiveness of the method, the drying processes of basic biopolymers with rigid rod shapes, such as polysaccharides, microtubules (MTs), and DNA, were investigated. We chose these biopolymers because they are typical examples of hierarchical macromolecules with megamolecular weights, and their intermolecular interactions enable them to form LC states.

Protocol

1. Instruments

1. **Polarization device**
    1. To construct a polarization device, provide a light source with a halogen lamp, a light guide, polarizers, a sample stage, an optical rail, rod stands, and a digital single-lens reflex camera (see **Figure 1C** and **Materials List** for the polarization device parts).

2. Preparation of the Biopolymer Solution

1. **Polysaccharide solutions**
    1. Dissolve sacran[7] (0.5 g) in pure water (100 mL) by stirring at ~80 °C for more than 12 h. During the dissolution, cover the container with plastic wrap to prevent evaporation. Prepare an aqueous solution of xanthan gum in the same manner.
    2. Cool the solutions at ~25 °C to obtain 0.5 wt% aqueous solutions.
    3. Centrifuge the sacran solution to remove impurities (48,400 x g, 4 °C, 1 h, 3 times).

2. **MT solution**
    1. Prepare a 0.5 wt% tubulin solution (1 mL) in a Britton-Robinson buffer (80 mM piperazine-*N,N*′-bis(2-ethanesulfonic acid) (PIPES); 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA); and 5 mM $MgCl_2$, pH 6.8) on ice[8].
    2. Use 0.5 wt% tubulin solution (50 µL) and guanosine-5'-[(α,β)-methyleno]triphosphate (GpCpp) (5 µL) to prepare a GpCpp-containing tubulin solution (50 µL). Incubate at 37 °C for 3 h to obtain a stable MT nucleus.
    NOTE: The role of GpCpp is to support MT formation and to completely suppress the depolymerization of MT to tubulin.
    3. Mix the 0.5 wt% tubulin solution (950 µL) and the GpCpp-containing tubulin solution (50 µL) at ~25 °C for 1 day to obtain a stable 0.5 wt% MT solution.

3. **DNA solution**
    1. Prepare a 0.5 wt% DNA solution (1 mL) in Tris-EDTA buffer solution (10 mM Tris, pH 8.0, with 1 mM EDTA).

4. Keep the 0.5 wt% biopolymer sample solutions at 25 °C for the drying experiment.

3. Drying Experiments and Observation under Cross-polarized Light

1. **Solutions in a one-side-open cell (Figure 1A)**
    1. Cut a silicon sheet (see Materials List) into an appropriate shape with a thickness of 1 mm (inner dimension of 5-15 mm, 1 mm, and ~20 mm; **Figure 1A**).
        1. Assemble a one-side-open cell composed of a silicon spacer with inner dimension of 5-15 mm, 1 mm, and ~20 mm and two non-modified glass slides (76 mm × 1 mm × 26 mm). Fix both sides of the cell with double clips in advance to keep the sample solution from leaking out.
    2. Slowly add each of the 0.5 wt% biopolymer solution (100-300 µL) using a ~1-mm pore-size pipette tip to each cell at ~25 °C. Remove air bubbles from the cells using a syringe needle.
    3. Place the cells in an oven with an air circulator at 60 °C under atmospheric pressure for evaporation; the evaporation direction is opposite to that of gravity.

2. **Observations under cross-polarized light (Figure 1B-1C)**
    1. Provide straight visible light via a 100 W halogen lamp with a flat-surface light source over a wide area (80 mm x 80 mm). Adjust the polarizers to 45° and 135° using the holders (**Figure 1C**).
    2. Fix the positions of the light source, polarizers, sample stage, and camera using an optical rail and rod stands (**Figure 1C**). Place the sample stage between the two polarizers (the distance between the polarizers should be ~5 cm). Place the camera ~20 cm from the sample stage to allow focusing.
    3. At given times, place the samples from step 3.1.3 between the polarizers on the stage parallel to the XZ-plane and cover the device with a black curtain; the actual device is shown in **Figure 1C**.
    4. Photograph the samples through linear crossed polarizers using a digital single-lens reflex camera with a standard zoom lens (see Materials List). Control the camera settings, such as focal distance, using computer software (see Materials List).

3. **Spatio-temporal analysis of the transmitted light intensity (Figure 1C)**
    1. To evaluate the change of the orientational order parameter in the drying process, collect photographs hourly for 24 h.
    2. Measure the transmitted light intensity along the centerline in the Z-direction as a gray value using an image processing program (*e.g.,* ImageJ).
    3. Plot a graph of the gray value as a function of the distance from the upper open side.

4. **Microscopic observations under cross-polarized light (Figure 1D)**
    1. To check the methods, make microscopic observations with a polarization microscope equipped with a CCD camera[9]. Keep a first-order retardation plate in the light path. Control the conditions for the photos using a PC software.

Representative Results

Using the device as shown in **Figure 1**, the self-integration from microdomain to macrodomain on a drying air-LC interface was evaluated (**Figure 2A**). As the first demonstration of the drying experiment, two kinds of megamolecular polysaccharides, sacran (M_w = 1.9 x 10^7 g mol^{-1}) and xanthan gum (4.7 x 10^6 g mol^{-1}), were compared. **Figure 2B** shows photographs of the solutions in the cell under cross-polarized light. Before drying, it was possible to observe several bright regions with transmitted light in a scattered state in both solutions. After drying at 60 °C for 6 h, the region beneath the air-LC interface in the sacran solution became significantly high. This means that the domains formed a macroscopically oriented structure at the interface, similar to skin layer formation in a gel-shrinking process[10]. On the other hand, the intensity of the xanthan solution decreased drastically when the temperature was just increased from 25 °C to 60 °C, and some small macrodomains were observed beneath the interface. This is because the mobility of the xanthan microdomain is much more sensitive to temperature than that of the sacran microdomain. The critical differences of the orientation on the interface were thus detected by using the polarization device.

Figure 2C and **Movie S1** show the results of spatio-temporal analysis for the drying process in the sacran solution. The transmitted light intensity indicates the orientational order parameter of the microdomain. The peak intensity around the air-liquid interface increased significantly, and the thickness grew to ~2 mm. These results clearly indicate that the microdomains start to orient from the air-LC interface and grow into a milliscale domain parallel to the interface. From this observation under polarized light, it is thus possible to visualize the fluid motions in the drying process.

The differences between sacran and xanthan gum in terms of macrodomain size and orientation direction were also confirmed by a polarization microscope with a first-order retardation plate (**Figure 2D**). The sacran fluid phase showed one blue region, meaning that a single macrodomain formed at the interface. In contrast, the xanthan gum fluid phase showed blue, yellow, and pink regions, meaning that the multiple macrodomains formed with arbitrary orientations.

This method was also explored for comparing three types of basic rigid biopolymers with megamolecular weights – polysaccharides (sacran: M_w = 1.9 x 10^7 g mol^{-1}, microdomain length >20 µm), MTs (M_w = 10^9-10^{10} g mol^{-1}, microdomain length >10 µm), and DNA (M_w = 1.3 x 10^6 g mol^{-1}, microdomain length <1 µm) – in a physiological environment at 37 °C. Before drying as shown in **Figure 3B**, the sacran solution and the MT solution showed similar LC states, with scattered domains in the whole area. During the drying process, the domains in the MT solution also underwent self-integration from the air-LC interface, and the scattered domains were integrated into a single macrodomain. On the other hand, the DNA solution showed no specific orientation in the liquid phase. In case of the MT solution and the DNA solution prepared with buffers, it is important to note that integration is affected by the changes in salt concentration, pH, and ionic strength during the drying.

To clarify the orientational order parameter and the directions of the adsorbed polymer films, the drying records were also observed by a polarization microscope with a first-order retardation plate (**Figure 3C**). The sacran drying record exhibited significant birefringence intensities because of the milliscale single macrodomain formation. For the MT drying record, we observed wavy bundles where the long axis was parallel to the X-axis. In contrast, the drying record of the DNA solution showed grain-shape macrodomains <5 µm in diameter and in arbitrary directions. From these observations, it is clear that the macrodomain size is affected by the length of the rod-like microdomains. It was thus possible to evaluate the directional orientation of the biopolymer microdomains by observing the drying records using a polarization microscope.

In conclusion, methods using polarized light were used for the self-integration of megamolecular biopolymers on the drying air-LC interface. The dynamics of the aqueous LC solutions were monitored by evaporating water from a one-side-open cell. By analyzing the images taken using cross-polarized light, it was possible to recognize the spatio-temporal changes and the orientational order parameter. Considering that the demonstrated drying process is similar to natural processes, this method would be useful for the characterization of not only artificial materials in various fields, but also of natural living tissues. We believe that this could provide an evaluation method for soft materials in the biomedical and environmental fields.

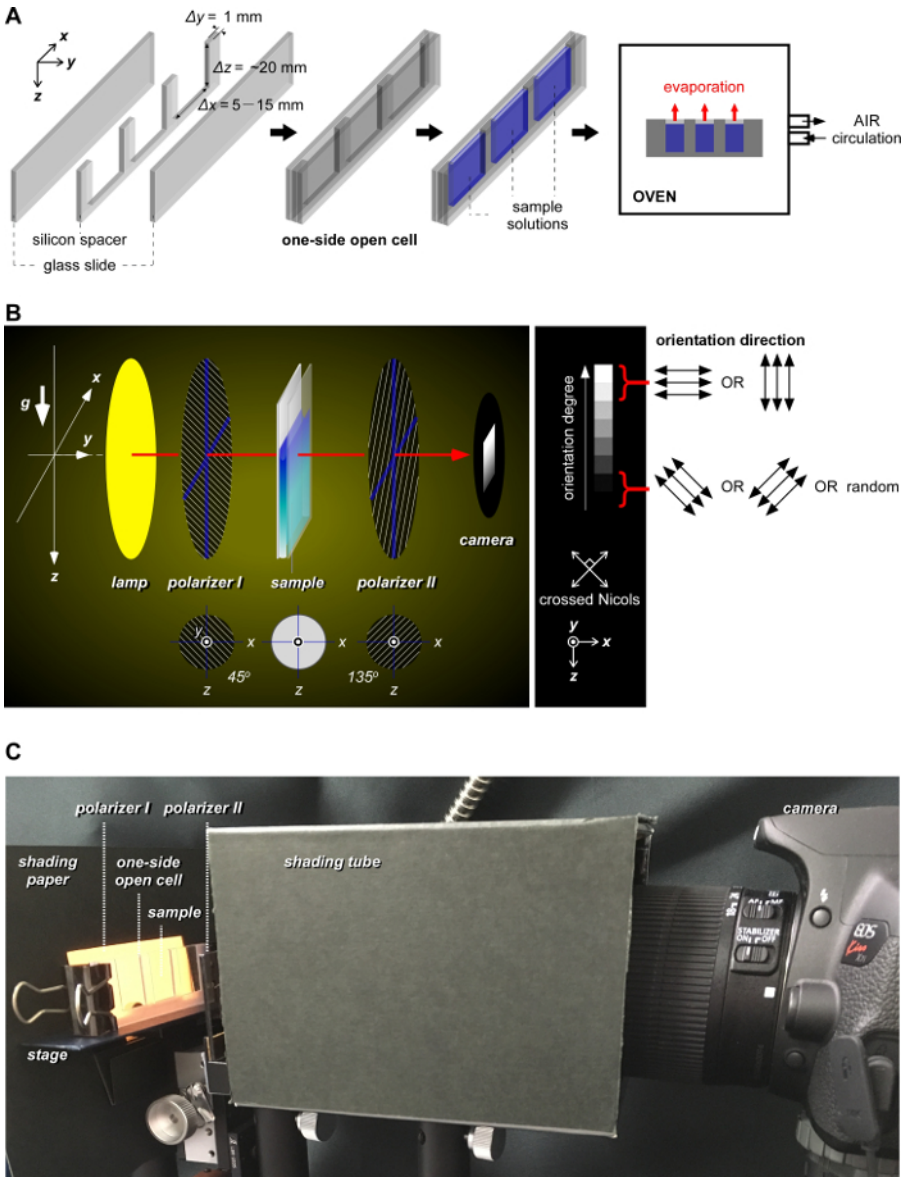


Figure 1: Drying experiment. (**A**) Schematic illustration of the solutions in a one-side-open cell. (**B**) Schematic illustration of the experimental device used for observations under cross-polarized light. The polarizers were normally adjusted to 45° and 135°. (**C**) The actual device. This figure has been modified from reference[6]. Copyright: The American Chemical Society, 2016. Please click here to view a larger version of this figure.

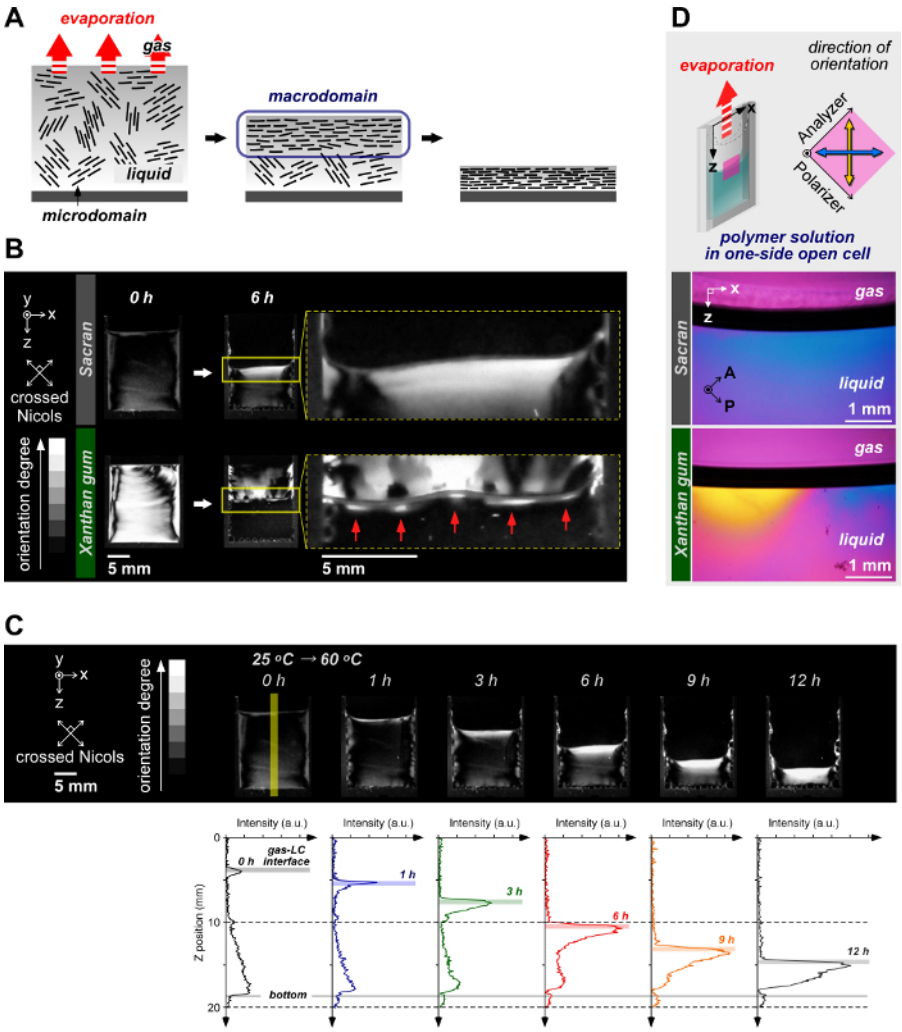


Figure 2: Drying process of aqueous solutions of the polysaccharides sacran and xanthan gum. (**A**) Schematic illustration of the self-integration on the drying air-LC interface. (**B**) Side views of two kinds of polysaccharide solutions at their initial states and after 6 h of drying at 60 °C under cross-polarized light. The initial polymer concentrations were 0.5 wt%. (**C**) Transmitted light intensity through a crossed Nicols on a line in the Z-direction for each picture taken at a given time. (**D**) Polarization microscopic images of the polymer solutions in the cell after 6 h of drying at 60 °C. Red arrows: macrodomains on the interface. This figure has been modified from reference[6]. Copyright: The American Chemical Society, 2016. Please click here to view a larger version of this figure.

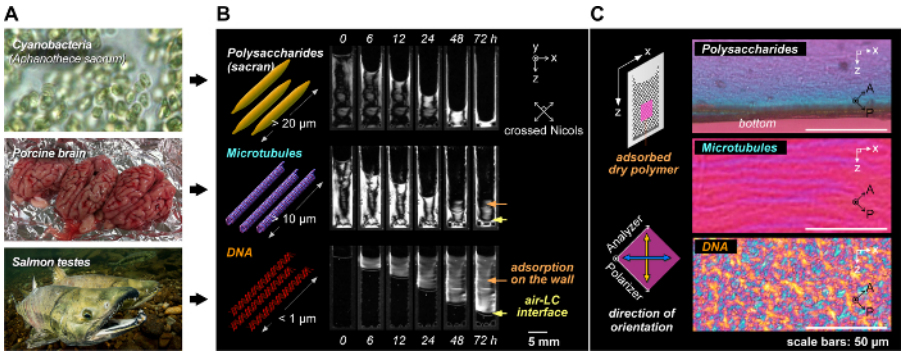
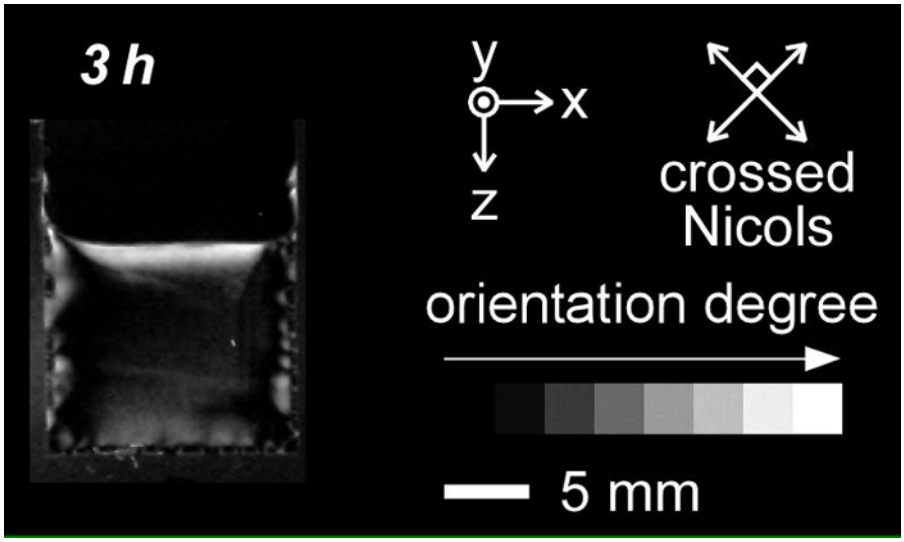


Figure 3: Drying process of biopolymer aqueous solutions and the drying records. (**A**) Sources of the polysaccharides (cyanobacteria), MTs (porcine brain), and DNA (salmon testes). (**B**) Side views of sacran, MT, and DNA solutions during drying at 37 °C under cross-polarized light. The initial polymer concentrations were 0.5 wt%. (**C**) Microscopic images of the dried polymer films on the glass substrate through the given directions of the crossed Nicols. All scale bars are 50 μm. This figure has been modified from reference[6]. Copyright: The American Chemical Society, 2016. Please click here to view a larger version of this figure.



Movie S1: Drying process of the sacran solution during drying at 60 °C, observed using crossed Nicols. Please click here to download this movie.

Discussion

It was sometimes difficult for the camera to focus on the sample due to the transmitted light intensity being too low. In such cases, placing an extended transparent plastic film on the stage helped to arrange the focus. The limitation of the observable resolution was dependent on the camera lens, ~10 μm in this case. The observable limitation of the sample thickness, Δy, was dependent on the maximum light intensity of the lamp, ~10 mm in this case.

The advantage of the device shown in **Figure 1B** is that it allows side-view photos of the sample to be taken during the drying process. The observation can be carried out without relying on the horizontal plane, which is used in typical microscopes. By standing the one-side-open cell during the drying experiments, the evaporation direction is regulated and in the opposite direction of gravity. Monitoring the side view also enables the calculation of the drying rate[4].

In the future, by placing temperature and humidity regulators on the sample stage of the device, it will be possible to automatically monitor temporal changes in the orientational order parameter. Furthermore, a balance in the setup to monitor the weight change would help the estimation of the concentration. This method could also be used to clarify the anisotropic swelling process of polysaccharide hydrogels[11,12]. It would therefore be possible to monitor the structural changes of soft actuators.

Disclosures

The authors have nothing to disclose.

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