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Imaging Trypsin Activity through Changes in the Orientation of Liquid Crystals Coupled to the Interactions between a Polyelectrolyte and a Phospholipid Layer

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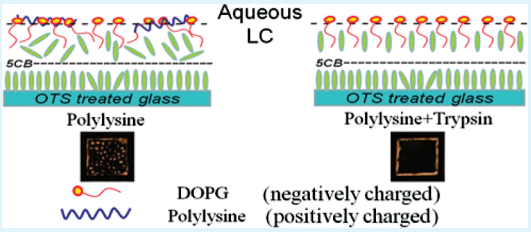
ABSTRACT: In this study, we developed a new type of liquid crystal (LC)-based sensor for the real-time and label-free monitoring of enzymatic activity through changes in the orientation of LCs coupled to the interactions between polyelectrolyte and phospholipid. The LCs changed from dark to bright after an aqueous solution of poly-L-lysine (PLL) was transferred onto a self-assembled monolayer of the phospholipid, dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), at the aqueous/LC interface. Interactions between the positively charged PLL and the negatively charged DOPG drove the reorganization of the phospholipid membrane, which induced an orientational transition in the LCs from a homeotropic to planar state. Since the serine endopeptidase trypsin can enzymatically catalyze the hydrolysis of PLL, the dark-to-bright shift in the optical response was not observed after transferring a mixed solution of PLL and trypsin onto the DOPG-decorated LC interface, indicating that no orientational transitions in the LCs occurred. However, the optical response from dark to bright was observed when the mixture in the optical cell was replaced by an aqueous solution of PLL. Control experiments with trypsin or an aqueous mixture of PLL and deactivated trypsin further confirmed the feasibility of this approach. The detection limit of trypsin was determined to be ~1 μg/mL. This approach holds great promise for use in the development of LC-based sensors for the detection of enzymatic reactions in cases where the biological polyelectrolyte substrates of enzymes could disrupt the organization of the membrane and induce orientational transitions of LCs at the aqueous/LC interface.



KEYWORDS: *liquid crystals, biosensors, orientational transitions, polylysine, trypsin, interface*

1. INTRODUCTION

During the past decade, liquid crystalline materials have drawn great attention for the sensitive amplification and transduction of chemical and biological events into optical outputs that are visible by the naked eye under crossed polarizers.[1−10] Since the orientation of liquid crystals (LCs) responds rapidly to analytes with high sensitivity and spatial resolution, the LC-based sensing technique may be a simple, effective, and promising approach for the detection of biomolecular interactions occurring at biological membranes.[11−13] Compared with traditional analytical methods, LC-based sensors have several significant advantages. They do not require the use of labeled molecules,[14] complex instrumentations,[15] or laborious techniques.[16]

Previous studies have shown that the orientational transition of LCs could be coupled to a variety of biomolecular interactions such as protein binding events and enzymatic reactions at fluid lipid-decorated interfaces between aqueous phases and thermotropic LCs.[11,12] For example, Brake et al. demonstrated that the spontaneous assembly of phospholipids at aqueous/LC interfaces resulted in a dark image in the optical response and specific binding of proteins to these lipid-decorated interfaces drove the reorganization of phospholipid membranes and caused orientational transitions of LCs, which gave rise to a change from dark to bright. Moreover, the transfer of phospholipase A_2 (PLA_2) onto the lipid-decorated interfaces led to a dark-to-bright shift in the optical response, because PLA_2 could catalytically hydrolyze the phospholipid and induce orientational transitions of the LCs.[11]

Several other strategies to monitor enzymatic reactions using LC-based sensors have been developed.[17−22] Park et al. reported that the oligopeptide-based polymeric membrane formed at the aqueous/LC interface retarded the transport of phospholipid to the LC interface, but the transport properties of the oligopeptide-decorated membrane were facilitated by incubating the membrane in an aqueous solution of enzymes that were specific for the oligopeptide substrates.[19] An orientational transition of LCs was obtained when the sample was treated with the enzymes, because the increased permeability of the polymeric membrane enhanced the transportation of phospholipid onto the LC interface after the enzymatic reactions were complete. In another study, a dark-to-bright shift in the optical response was observed after immobilizing a 17-amino-acid oligopeptide onto the lipid-decorated LC interface and transfer of enzymes to the oligopeptide-decorated interface caused selective hydrolysis of

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the oligopeptide, which resulted in the reorganization of the lipid membrane at the interface.[20] Thus, the orientation of LCs changed and the optical response reverted from bright to dark.

We recently developed a new method to monitor molecular interactions between antimicrobial agents and lipid membranes using LCs.[23] An optical response from bright to dark was observed when a positively charged antimicrobial agent was in contact with the LC interface decorated with a self-assembled monolayer of negatively charged phospholipid. This approach was shown to hold great promise in studying biological events such as membrane disruptions and enzymatic reactions.

In this study, we developed a new type of LC-based sensor to monitor enzymatic reactions based on interactions between the positively charged biological polyelectrolyte substrates of enzymes and the negatively charged lipid membrane decorated at aqueous/LC interfaces. We predicted that these interactions can lead to the reorganization of phospholipid membranes and cause a planar orientation of LCs at the interface (see Figure 1a). In contrast, the presence of enzymes specific for the

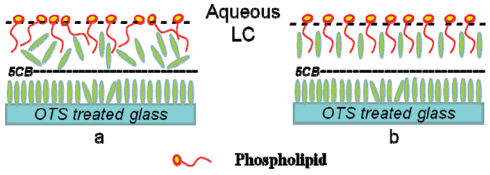

Figure 1. Schematic illustration of the orientation of LCs associated with different organizations of phospholipid membranes at the aqueous/LC interface: (a) planar orientation and (b) homeotropic orientation.

polyelectrolyte substrates may weaken or eliminate these interactions, which would cause the orientation of the LCs to remain in a homeotropic state (see Figure 1b). Since trypsin, which catalyzes the hydrolysis of amide linkages and esters of lysine, is an important digestive enzyme produced by the pancreas,[24,25] enzymatic reactions of trypsin and poly-L-lysine (PLL) (mol wt 150 000−300 000) were used to confirm the feasibility of our prediction. We studied orientational behaviors of LCs after contacting PLL with LC interfaces decorated with the self-assembled monolayer of the phospholipid, dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG) and examine the orientation of LCs after transferring PLL that had been pretreated with trypsin onto DOPG-decorated LC interfaces. This novel approach holds great promise for imaging the activity of enzymes coupled to the interactions between the biological polyelectrolyte and the lipid membrane decorated at the aqueous/LC interface.

2. EXPERIMENTAL SECTION

2.1. Materials. Nematic liquid crystal 4-cyano-4′-pentyl-biphenyl (5CB), manufactured by BDH, was purchased from EM industries (Hawthorne, NY). The premium glass microscope slides were obtained from Fisher Scientific (Pittsburgh, PA). Copper specimen grids (50 mesh, pitch = 508 μm, hole = 425 μm, bar = 83 μm) were purchased from GILDER. Sulfuric acid, hydrogen peroxide (30% w/v), octyltrichlorosilane (OTS), 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), lysine, poly-L-lysine (PLL) (mol wt 150 000−300 000) (0.1% w/v), trypsin, and phosphate buffered saline (PBS) (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl; pH 7.4), were purchased from Sigma−Aldrich. All aqueous solutions were prepared with deionized water (18 MΩ cm), using a Milli-Q water purification system (Millipore, Bedford, MA). The pH of the PBS solution was adjusted to 7.6 prior to use.

2.2. Treatment of Glass Microscope Slides with OTS. OTS-treated glass slides were prepared following procedures detailed in previous publications.[18,23] Briefly, the glass microscope slides were cleaned using a "piranha solution" (70% H_2SO_4/30% H_2O_2 for 30 min at 80 °C. *[Caution: "piranha solution" reacts violently with organic materials and should be handled with extreme caution; do not store the solution in closed containers.]* The slides were then rinsed with water, ethanol, and methanol, and dried under a stream of gaseous N_2, followed by heating to 120 °C overnight prior to OTS deposition. The "piranha-cleaned" glass slides were immersed into an OTS/*n*-heptane solution for 30 min. The slides were then rinsed with methylene chloride and dried under a stream of N_2.

2.3. Preparation of Optical Cells. Copper specimen grids were placed onto the OTS-treated glass slide that was fixed under an eight-well chamber slide. Then, 1.5 μL of 5CB, heated to its isotropic phase (>35 °C), was dispensed onto each grid, and the excess LC was removed by contacting a 20-μL capillary tube with the 5CB droplet on the grid. Subsequently, 400 μL aqueous solutions of interest were introduced into the optical cell at room temperature. Each assay was performed at least six times independently.

2.4. Formation of the Self-Assembled Monolayer of Phospholipids. LCs laden with a phospholipid monolayer were prepared following procedures published in previous literature.[3,4,12,23] DOPG dissolved in chloroform was dried with N_2 gas and placed under vacuum for at least 3 h. The dried phospholipid then was resuspended with PBS to a final concentration of 1 mM. Next, the phospholipid suspension was sonicated three times each for 5 min at room temperature to obtain a clear solution. Subsequently, the solution was filtered twice using a 0.22-μm filter and typically used within 1 day after the preparation. The phospholipid monolayer was formed by contacting the copper grid impregnated with 5CB to the phospholipid solution in the optical cell.

2.5. Preparation of Aqueous Solutions. All aqueous solutions were prepared in PBS (pH 7.6). Briefly, PLL solutions with different concentrations were prepared by diluting the original PLL solution (0.1% w/v) in PBS. The deactivated trypsin was obtained by incubating trypsin solution in an oven for 30 min at a temperature of 70 °C. Aqueous mixtures of PLL and trypsin or deactivated trypsin were incubated for 1 h at the room temperature before they were introduced into the optical cell.

2.6. Optical Examination of LC Textures. Imaging the optical appearance of the LC was made with a digital camera (DS-2Mv, Nikon, Tokyo, Japan) attached to a polarized light microscope (ECLIPSE LV100POL, Nikon, Tokyo, Japan).[18,23] The optical textures were formed by polarized light transmitted through optical cells filled with nematic 5CB. All images were obtained using a 4× objective lens between crossed polarizers; these images were captured at a resolution of 1600 × 1200 pixels, a gain of 1.00×, and a shutter speed of 1/10 s.

3. RESULTS AND DISCUSSION

3.1. Imaging Interactions between Poly-L-lysine and Phospholipids at the Aqueous/LC Interface. In order to build a detection system for monitoring the enzymatic activity of trypsin, we first studied the optical response of LCs coupled to the interactions between the positively charged PLL (mol wt 150 000−300 000) and the negatively charged phospholipid at aqueous/LC interfaces. A change in the optical response from bright (Figure 2a) to dark (Figure 2b) was observed after an aqueous solution of 1 mM DOPG in PBS (pH 7.6) was transferred onto the LC interface, which represents that an orientational transition of the LCs from a planar to a homeotropic state occurred. The DOPG solution was incubated for 30 min in the optical cell to form a stable self-assembled monolayer of phospholipid at fluid interfaces.

Next, we exchanged the aqueous solution of phospholipid with PBS five times to remove free lipid from the bulk solution and then added an aqueous solution of 0.01% (w/v) lysine in

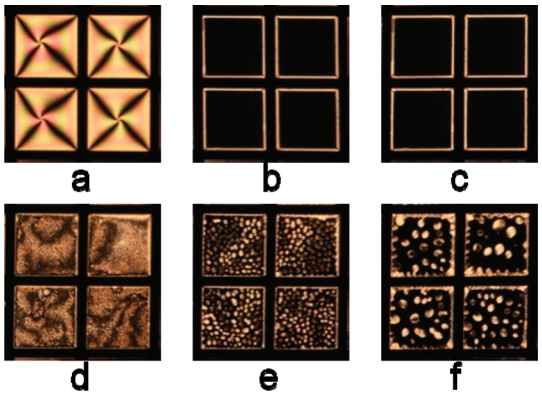

Figure 2. Polarized light microscopy images of 5CB: (a) immersed in deionized (DI) water, (b) in contact with DOPG in PBS, (C) incubated with 0.01% (w/v) lysine monomers in PBS, (d) 30 s after contacting the DOPG-decorated interface with 0.01% (w/v) PLL, (e) 90 s after incubating the DOPG-decorated interface with 0.01% (w/v) PLL, and (f) 30 min after immersing the DOPG-decorated interface in 0.01% (w/v) PLL.

PBS into the optical cell. The LC remained dark (Figure 2c) during 1 h of observance, suggesting a homeotropic alignment of LCs. This result shows that incubation of lysine monomers with the DOPG-decorated LC interface could not lead to an orientational transition of LCs. Thus, no distinctive change was observed in the optical response.

We then examined the optical response of LCs after transferring an aqueous solution of 0.01% (w/v) PLL onto the LC interface decorated with the negatively charged phospholipid, DOPG. A change in the optical response from dark to bright (Figure 2d) was obtained within 1 min after the addition of PLL, indicating that the LCs underwent an orientational transition from a homeotropic state to a planar state. We also observed the time-dependent nucleation (Figure 2e) and growth (Figure 2f) of bright domains in LCs, which might be coupled to the dynamic lateral distribution of PLL-DOPG complex at fluid interfaces. These results were consistent with our previous study that molecular interactions between positively charged antimicrobial agents could disrupt the organization of negatively charged phospholipid membranes and result in the orientational transition of LCs at the aqueous/LC interface.[23]

3.2. Monitoring the Enzymatic Reaction of Trypsin with PLL. Since trypsin can cleave PLL into small peptide fragments, we predicted that the dark-to-bright shift in the optical response of LCs may not occur after an aqueous mixture of trypsin and PLL was introduced onto the LC interface decorated with DOPG. We first tested the optical response of LCs after transferring an aqueous solution of 0.0005% (w/v) PLL onto the DOPG-decorated LC interface. The optical response changed from dark (Figure 3a) to bright (Figure 3b) after the addition of the PLL solution into the optical cell, indicating that the LCs underwent an orientational transition from a homeotropic state to a planar state. We then introduced an aqueous mixture of 0.1 mg/mL trypsin and 0.0005% (w/v) PLL to the DOPG-decorated interface. The aqueous mixture was incubated for 1 h at room temperature before it was transferred to the optical cell. Under these conditions, the LCs remained dark (Figure 3c) for 30 min, which indicates that the orientation of LCs did not change. Next, we exchanged the aqueous mixture with PBS to wash the interface and introduced

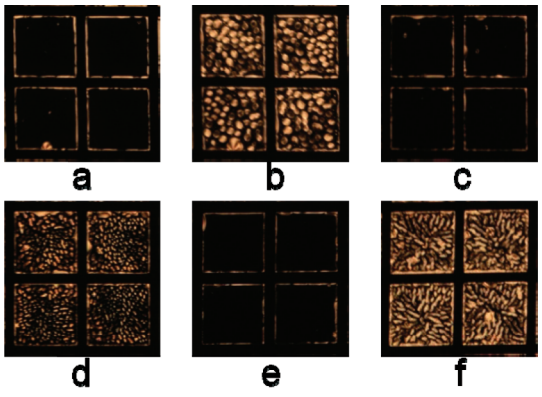

Figure 3. Optical images of 5CB: (a) decorated with DOPG, (b) 3 min after immersing the DOPG-decorated interface in 0.0005% (w/v) PLL, (c) 30 min after incubating the DOPG-decorated interface with an aqueous mixture of 0.1 mg/mL trypsin and 0.0005% (w/v) PLL, (d) 3 min after transferring 0.0005% (w/v) PLL onto the DOPG-decorated interface pre-incubated with the aqueous mixture, (e) 30 min after immersing the DOPG-decorated interface with 0.1 mg/mL trypsin, and (f) 3 min after incubating the DOPG-decorated interface in an aqueous mixture of 0.1 mg/mL deactivated trypsin and 0.0005% (w/v) PLL.

an aqueous solution of 0.0005% (w/v) PLL onto the same DOPG-decorated LC interface. When this was done, the LCs changed from dark to bright (Figure 3d) within 3 min, which demonstrates that the orientation of the LCs changed from a homeotropic state to a planar state. These observations suggest that the enzymatic reaction of trypsin and PLL could be monitored through the interactions between the positively charged PLL and the negatively charged DOPG at the aqueous/LC interface.

To further confirm the feasibility of using this system to monitor the enzymatic reaction between trypsin and PLL, we conducted two control experiments. First, an aqueous solution of 0.1 mg/mL trypsin in PBS was transferred onto the DOPG-decorated LC interface. After transfer, the LC remained dark (Figure 3e) for 30 min, which indicates that the addition of trypsin solution did not induce an orientational transition of LCs. Second, we immersed the DOPG-decorated LC interface into an aqueous mixture of 0.1 mg/mL deactivated trypsin and 0.0005% (w/v) PLL. When this was done, the LC became bright (Figure 3f) within 3 min, suggesting that the LC transitioned into a planar orientation. The trypsin was deactivated by incubating the trypsin solution in an oven at a temperature of 70 °C for 30 min. According to a previous study,[26] trypsin could be completely deactivated under such a condition. Before transferring the aqueous mixture, we also incubated a mixed solution of trypsin and PLL for 1 h at room temperature. Based on these results, we further concluded that the enzymatic reaction of trypsin and PLL could be monitored using this system.

3.3. Detection Limit of the Enzymatic Activity of Trypsin. Since the enzymatic reaction of trypsin and PLL can be monitored using the experimental system described above, we next examined the enzymatic activity of trypsin in more detail. First, we tested the detection limit of PLL to obtain an optimal concentration for the determination of trypsin activity in real-time and with high spatial resolution. In these experiments, the LCs appeared bright within 5 min after the transfer of an aqueous solution of 0.0002% (w/v) PLL onto the

DOPG-decorated LC interface (Figure 4a). LCs exhibited a partially bright appearance (Figure 4b) after immersing the

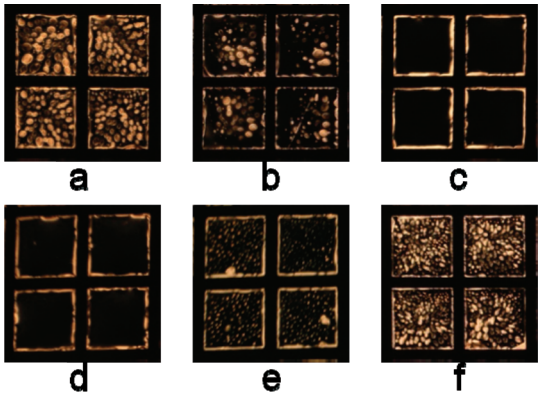

Figure 4. Polarized light microscopy images of 5CB: (a) 5 min after the DOPG-decorated interface was in contact with 0.0002% (w/v) PLL, (b) 30 min after incubating the DOPG-decorated interface in 0.0015% (w/v) PLL, (c) 2 h after immersing the DOPG-decorated interface in 0.0001% (w/v) PLL, (d) 30 min after transferring an aqueous mixture of 10 μg/mL trypsin and 0.0002% (w/v) PLL onto the LC interface decorated with DOPG, (e) 30 min after the DOPG-decorated interface was in contact with an aqueous mixture of 1 μg/mL trypsin and 0.0002% (w/v) PLL, and (f) 3 min after incubating the DOPG-decorated interface with an aqueous mixture of 0.1 μg/mL trypsin and 0.0002% (w/v) PLL.

DOPG-decorated LC interface in an aqueous solution of 0.00015% (w/v) PLL for 30 min. However, the LC remained dark (Figure 4c) after incubating the DOPG-decorated LC interface with an aqueous solution of 0.0001% (w/v) PLL for 2 h. In view of these results, we used a PLL solution with a concentration of 0.0002% (w/v) to determine the enzymatic activity of trypsin.

When an aqueous mixture of 10 μg/mL trypsin and 0.0002% (w/v) PLL pre-incubated for 1 h was transferred onto the DOPG-decorated LC interface, the LCs remained dark (Figure 4d) for 30 min, indicating that the orientation of LCs remained in a homeotropic state after the enzymatic reaction between trypsin and PLL. Next, we introduced a mixed solution of 1 μg/mL trypsin and 0.0002% (w/v) PLL to the LC interface decorated with DOPG, and only some small bright domains (Figure 4e) were formed after 30 min, which demonstrates that the PLL concentration was decreased after the enzymatic reaction. We also immersed the DOPG-decorated LC interface into an aqueous mixture of 0.1 μg/mL trypsin and 0.0002% (w/v) PLL and the optical response of LCs changed from dark to bright (Figure 4f) within 3 min, indicating that the LCs underwent an orientational transition from a homeotropic state to a planar state. This observation suggests that the concentration of the PLL was not greatly decreased after the enzymatic reaction of trypsin and PLL. Thus, the PLL was still sufficient to induce a dark-to-bright shift in the optical response of LCs. These results suggest that the detection limit of trypsin in this system was ~1 μg/mL. We predict that the detection limit could be lowered by extending the incubation time of the aqueous mixture before it is transferred onto the DOPG-decorated LC interface.

4. CONCLUSIONS

In summary, we have developed a new method to monitor the enzymatic activity of trypsin using liquid crystals (LCs). The transfer of an aqueous solution of positively charged poly-L-lysine (PLL) onto the negatively charged dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG)-decorated LC interface induced a change in the optical response from dark to bright. The introduction of a mixed solution of trypsin and PLL to the interface decorated with DOPG did not produce an optical response, but the LC did undergo a dark-to-bright shift after PLL was transferred to the same DOPG-decorated interface. We also investigated the optical response of LCs when aqueous solutions of PLL at different concentrations were transferred onto the DOPG-decorated interface and the detection limit of trypsin. This approach holds great promise for use in detecting the activity of enzymes coupled to the interactions between the biological polyelectrolyte and lipid membranes decorated at the aqueous/LC interface.

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Notes

The authors declare no competing financial interest.

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