Talanta 99 (2012) 36–39

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A simple strategy to monitor lipase activity using liquid crystal-based sensors

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ARTICLE INFO

Article history:
Received 6 April 2012
Received in revised form
7 May 2012
Accepted 9 May 2012
Available online 16 May 2012

Keywords:
Liquid crystals
Sensors
Lipase
Glyceride
Carboxylate monolayers
Interface

ABSTRACT

In this study, we developed a simple label-free technique for monitoring the enzymatic activity of lipase using liquid crystal (LC)-based sensors. The optical response of LCs changed from a bright to dark appearance when an aqueous solution of lipase was in contact with a nematic LC, 4-cyano-4′-pentylbiphenyl (5CB), that was doped with glyceryl trioleate, which is a glyceride that can be enzymatically hydrolyzed by lipase. Since the oleic acid released from the enzymatic reaction could spontaneously form a self-assembled monolayer at the aqueous/LC interface due to its amphiphilic property, the orientation of the LCs transited from a planar to homeotropic state, which induced a change in the optical response of the LCs. We did not observe a bright-to-dark shift in the optical appearance of LCs when pure 5CB was immersed into the lipase solution. Moreover, we further confirmed the specificity of the enzymatic reaction by transferring an aqueous buffer solution not containing an analyte, or with bovine serum albumin (BSA) or trypsin onto the interface of aqueous solutions and the glyceryl trioleate-doped 5CB, which did not produce any distinctive contrast in the optical appearance. These results suggest the feasibility of measuring the enzymatic activity of lipase using the LC-based sensing technique. Furthermore, our strategy could also be used for the preparation of a self-assembled monolayer of carboxylates at the aqueous/LC interface.

1. Introduction

Lipases (triacylglycerol acylhydrolase; EC 3.1.1.3) are ubiquitous enzymes that catalyze the hydrolysis of triacylglycerols to diacylglycerols, monoacylglycerols, fatty acids and glycerol at the interface between an insoluble substrate and the aqueous phase [1–3]. They are widespread in many animals, plants, fungi and bacteria, and have promising applications in food, detergents, cosmetics, organic chemical processing, and pharmaceutical processing [3–6]. To date, a variety of approaches have been reported to monitor the presence of lipase, such as titrimetry [7], fluorimetry [8], colorimetry [9], and spectrometry [10]. In particular, the determination of lipase activity in biological fluids is tightly coupled to the clinical diagnosis of human diseases [4,11]. However, all existing methods have certain limitations; thus, it is very important to develop new strategies to address these issues [11].

During the past decade, liquid crystal (LC)-based sensors have shown promise for the amplification and transduction of chemical and biological interactions, which take place at surfaces or fluid interfaces, into optical signals visible by the naked eye [12–20]. For example, Gupta et al. demonstrated the recognition of ligand–receptor binding of proteins on surfaces with nanometer-scale topographies [12]. The specific binding event between proteins and immobilized ligands eliminate the surface topographies, which changed the intensity of the transmitted light coupled to the orientation of LCs. On the other hand, Brake et al. developed an alternative system to investigate a range of dynamic molecular phenomena at the interface between LCs and aqueous phases [13]. Strong and weak specific binding events induced reorganization of phospholipids that were assembled at fluid interfaces, resulting in the orientational transition of LCs that was readily imaged with polarized light. These LC-based analytical approaches are quite simple and do not require labeling of the analyte, complex instruments, or laborious techniques.

Recently, several studies have been published on building new types of sensors employing LCs doped with functional materials at fluid interfaces [21–23]. For instance, Bi et al. designed a real-time pH sensor by mixing a nematic LC, 4-cyano-4′-pentylbiphenyl (5CB), with 4′-pentyl-biphenyl-4-carboxylic acid (PBA) [21]. Due to the release of H^+ from the enzymatic reaction between surface-immobilized penicillinase and penicillin in the aqueous phase, the orientational transition of LCs was triggered at the aqueous/LC interface. In addition, we previously developed a simple label-free approach to detect catalase using dodecanal-doped 5CB [22]. Orientational transitions of LCs were obtained after transferring an aqueous solution of hydrogen peroxide onto

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http://dx.doi.org/10.1016/j.talanta.2012.05.016

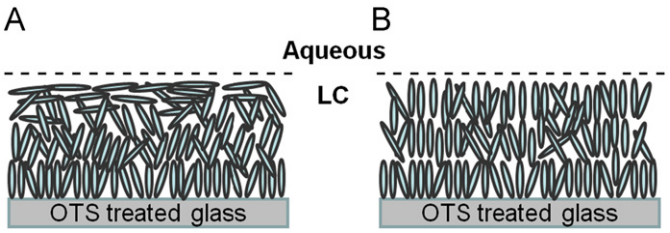

Fig. 1. Schematic illustration of the orientation of LCs before and after the enzymatic reaction between lipase and glyceryl trioleate: (A) planar orientation, (B) homeotropic orientation.

the aqueous/LC interface, because of the formation of self-assembled monolayers after aldehyde was oxidized into carboxylic acid. This phenomenon could not be observed when hydrogen peroxide was hydrolyzed by catalase via the enzymatic reaction in aqueous solutions, which provided a new strategy to recognize biological interactions associated with hydrogen peroxide.

Herein, we developed a simple label-free method to monitor the enzymatic activity of lipase at fluid interfaces. Due to the release of carboxylic acid from the enzymatic hydrolysis of glyceryl trioleate doped in 5CB by lipase at the aqueous/LC interface, we predict that a stable self-assembled monolayer of oleate may form and induce a transition in the orientation of LCs from a planar (Fig. 1A) to homeotropic (Fig.1 B) state, which can result in a change in the optical appearance of the LC from bright to dark. Compared with all the existing analytical techniques, this approach is quite simple and holds great promise for the determination of lipase activity.

2. Material and methods

2.1. Materials

Nematic liquid crystal 4-cyano-4′-pentylbi-phenyl (5CB), manufactured by BDH, was purchased from EM industries (Hawthorne, NY). Premium glass microscope slides were obtained from Fisher Scientific (Pittsburgh, PA). Gold specimen grids (75 meshes) were purchased from Electron Microscopy Science. Sulfuric acid, hydrogen peroxide (30% w/v), octyltrichlorosilane (OTS), lipase (from porcine pancreas), glyceryl trioleate, bovine serum albumin (BSA), trypsin (from porcine pancreas), tris buffered saline (TBS) (50 mM tris, 138 mM NaCl, 2.7 mM KCl; pH=8.0), calcium chloride dihydrate, 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).

2.2. Treatment of glass microscope slides with OTS

The glass microscope slides were cleaned using a "piranha solution" (70% H_2SO_4/30% H_2O_2, Caution: "piranha solution" reacts violently with organic materials and should be handled with extreme caution; do not store the solution in closed containers) for 30 min at 80 °C. 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

The OTS-coated glass slides were fixed at the bottom of an eight-well chamber slide. Gold specimen grids were then placed onto the OTS-coated glass slide. 2.0 μL of glyceyl trioleate-doped 5CB (1:30 (v/v)) was dispensed onto each grid after the LC was heated to temperatures that induced formation of the isotropic state. Excess LC was removed by placing the grid in contact with a 20 μL capillary tube containing a droplet of 5CB. This procedure led to the formation of a stable film of 5CB within the grid. Subsequently, the optical cell was heated to 50 °C again and then immediately immersed in aqueous solutions of interest at room temperature. All aqueous solutions were prepared in 50 mM tris buffered saline (TBS) with 5 mM calcium chloride, and the pH of the buffer solution was adjusted to 7.7 prior to use.

2.4. Optical examination of LC textures

A polarized light microscope (ECLIPSE LV100POL, Nikon, Tokyo, Japan) was used to image the optical textures formed by the polarized light transmitted through the optical cells filled with nematic 5CB. All images were obtained using a 4 × objective lens between crossed polarizers at room temperature. The optical response of the LC was imaged using a digital camera (DS-2Mv, Nikon, Tokyo, Japan) that was attached to the polarized light microscope. The 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 the enzymatic event of lipase at the aqueous/LC interface

In order to build a simple sensing system to monitor the enzymatic event of lipase, we first examined the optical response of 5CB doped with glyceryl trioleate in a buffer solution. The glyceryl trioleate-doped LCs exhibited a bright appearance (Fig. 2A) when an aqueous solution of TBS containing 5 mM calcium chloride was introduced into the optical cell, which indicates a planar orientation of the LCs at the aqueous/LC interface. In addition, pure 5CB also showed a bright appearance (data not shown) in the buffer solution. These results suggest that glyceryl trioleate may not induce an orientational transition of 5CB. Thus, there was no change in the optical appearance of the LCs. Next, we replaced the buffer solution with an aqueous solution of 0.1 mg/mL lipase in TBS containing 5 mM calcium chloride. Under these conditions, the optical response gradually changed from bright to dark (Fig. 2B), and became almost completely dark (Fig. 2C) within 1 h, implying an orientational transition of LCs from a planar to homeotropic state. We also found that the bright-to-dark change in the optical response was not uniform, which might be attributed to the nonuniform distribution of glyceryl trioleate at the aqueous/LC interface.

Previously, Abbott's group reported that the adsorption of surfactants, such as sodium dodecyl sulfate (SDS) in an aqueous phase, could induce the homeotropic alignment of LCs at fluid

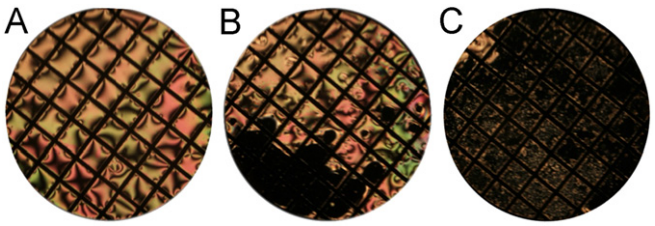

Fig. 2. Polarized light microscopy images of LCs after transferring an aqueous solution of 0.1 mg/mL lipase onto the interface of glyveryl trioleate-doped 5CB: (A) 0 h, (B) 0.5 h, (C) 1 h.

interfaces [24]. Hydrophobic interactions between the tail of surfactants and LC molecules played a dominant role in determining the homeotropic orientation of LCs. In addition, Fletcher et al. reported that 5CB underwent homeotropic alignment when it was in contact with an aqueous solution of 0.5 mM oleate at fluid interfaces [25]. Furthermore, they also found that the orientational behavior of oleic acid-doped 5CB was highly associated with the pH and ionic strength of the aqueous phase, which largely affected the areal density of surfactants absorbed at the interface. Once the areal density of amphiphiles surpassed a critical value, the oleic acid-doped 5CB underwent a change in orientation and adopted a homeotropic alignment.

Based on these previous studies, we examined the mechanism of the enzymatic event of lipase at the aqueous/LC interface. Due to the hydrophobic property of glyceryl trioleate, it could not spontaneously form a self-assembled monolayer at the interface of LCs and aqueous buffer solutions. Therefore, LCs adopted a planar alignment, and thus exhibited a bright appearance. After exchanging the buffer solution with an aqueous solution of lipase, enzymatic reactions occurred at the interface of the aqueous solutions and glyceryl trioleate. Due to the amphiphilic property of the released oleic acid, a self-assembled monolayer of surfactants was formed at fluid interfaces. Once the areal density of absorbed amphiphiles surpassed a critical value, the LCs transited from a planar to homeotropic state, and induced a bright-to-dark shift in the optical response of LCs. Although the distribution of glyceryl trioleate at the interface might be not uniform, due to the diffusion of the generated oleic acid from the glyceryl trioleate-laden area to the glyceryl trioleate-free area, the LCs appeared uniformly black. These results suggest that the enzymatic event of lipase may be investigated using the glyceryl trioleate-doped 5CB.

3.2. The specificity of the enzymatic reaction

Several control experiments were performed to further confirm that the bright-to-dark shift in the optical response and the planar-to-homeotropic transition in the orientation of glycryl trioleate-doped 5CB were due to the enzymatic reaction between lipase and glyceryl trioleate at the aqueous/LC interface. First, in order to determine whether lipase could affect the orientation of pure 5CB, we examined the orientational behavior of pure 5CB in contact with an aqueous solution of 0.1 mg/mL lipase in TBS containing 5 mM calcium chloride. In these experiments, the LCs remained bright in appearance (Fig. 3) over 4 h of observation, which suggests that the LCs adopted a planar orientation at the aqueous/LC interface. Thus, in the absence of glyceryl trioleate, the lipase did not induce an orientational transition of the LCs from a planar to homeotropic state. Subsequently, we examined the orientational behaviors of glyceryl trioleate-doped 5CB immersed in aqueous buffer solutions in the absence of an analyte, or in the presence of bovine serum albumin (BSA) or trypsin to confirm the specificity of the enzymatic reaction between lipase and glyceryl trioleate. The optical responses of these samples are shown in Fig. S1. We found that, during 4 h of observance, the optical response of glyceryl trioleate-doped 5CB immersed in the aqueous solution of TBS containing 5 mM calcium remained bright in appearance (Fig. S1A), which implies that LCs adopted a planar orientation at the aqueous/LC interface. In addition, the glyceryl trioleate-doped 5CB when in contact with aqueous solutions of 0.1 mg/mL BSA (Fig. S1B) or 0.1 mg/mL trypsin (Fig. S1C) in TBS containing 5 mM calcium chloride remained bright in appearance. Therefore, we determined that aqueous solutions of buffer, BSA, and trypsin did not induce an orientational transition of the glycery trioleate-doped 5CB. All of these results suggest that the planar-to-homeotropic transition in the orientation of LCs was due to the enzymatic reaction between lipase and glyceryl trioleate doped in 5CB, which indicates the specificity of the enzymatic event.

Fig. 3. Optical images of LCs after incubating pure 5CB with an aqueous solution of 0.1 mg/mL lipase: (A) 0 h, (B) 2 h, (C) 4 h.

3.3. The detection limit of lipase

After confirming the specificity of the enzymatic reaction between lipase and glyceryl trioleate at the aqueous/LC interface, we examined the detection limit of the experimental system for lipase. In order to determine the sensitivity of the detection system, the glyceryl trioleate-doped 5CB was immersed in aqueous solutions with different concentrations of lipase dissolved in TBS containing 5 mM calcium chloride. First, an aqueous solution of 10 μg/mL lipase was transferred onto the interface of glyceryl trioleate-doped 5CB. Under these conditions, the optical response began to change from bright (Fig. 4 A) to dark (Fig. 4B) in a single mesh of the gold grid within 1 h, which indicates that the LC underwent a planar-to-homeotropic transition at the fluid interface. Moreover, more than half of the meshes of the gold grid became dark within 2 h (Fig. 4C), which became uniformly dark (data not shown) within 3 h. Next, we immersed glyceryl trioleate-doped 5CB into an aqueous solution of 1 μg/mL lipase. The LCs confined in a single mesh of the gold grid began to undergo a bright (Fig. 4D)-to-dark (Fig. 4E) shift in optical response within 3 h. In addition, the number of meshes holding the LCs that exhibited a dark appearance increased with the incubation time, and the LCs appeared completely dark (data not shown) after incubation overnight. Furthermore, we also found that the optical response of LCs remained dark for more than 36 h, which demonstrated that the self-assembled monolayer of amphiphiles was quite stable at the aqueous/LC interface. Our results also demonstrate that the optical responses of the LCs were highly associated with the concentration of the lipase solution. The decrease in the lipase concentration would extend the time required for the orientational transition of LCs from

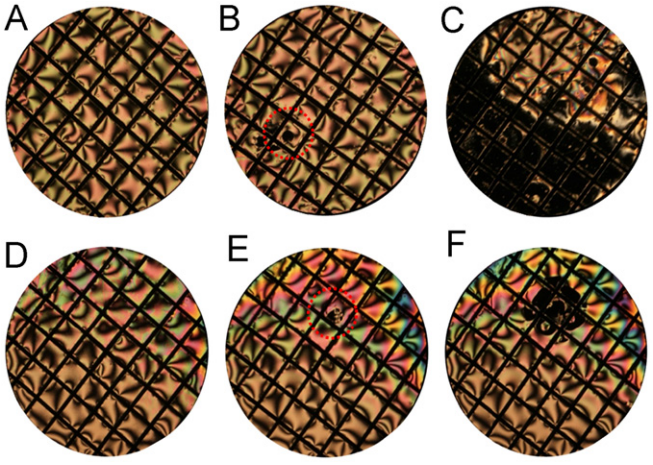

Fig. 4. Polarized light microscopy images of glyceryl trioleate-doped 5CB: immersed in an aqueous solution of 10 μg/mL lipase for (A) 0 h, (B) 1 h, and (C) 2 h; incubated with an aqueous solution of 1 μg/mL lipase for (D) 0 h, (E) 3 h, and (F) 6 h. The dark appearance is indicated within the center of the red circles in (B) and (E).

planar to homeotropic state. The detection limit of this LC-based lipase sensor was determined to be at least 1 μg/mL.

4. Conclusions

In summary, we developed a simple label-free method to determine the enzymatic activity of lipase using glyceryl trioleate-doped 5CB. The released oleic acid from the enzymatic reaction between lipase and glyceryl trioleate formed a self-assembled monolayer of surfactants at the aqueous/LC interface, which induced an orientational transition of the LCs from a planar to homeotropic state. Thus, the optical response of the LCs changed from bright to dark. Control experiments further confirmed the feasibility of this approach to monitor lipase activity. In addition, the detection limit of this system was at least 1 μg/mL. These results suggest that LC-based sensors hold great promise for the simple determination of lipase activity.

Acknowledgments

This work was supported by the Gachon University Research Fund in 2012 (GCU-2012-R069).

Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2012.05.016.

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