Colloids and Surfaces B: Biointerfaces 147 (2016) 100–105

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

A liquid crystal-based sensor for the simple and sensitive detection of cellulase and cysteine

Yi Wang [a], Qiongzheng Hu [b], Tongtong Tian [a,c], Yan'an Gao [d], Li Yu [a,*]

[a] Key Laboratory of Colloid and Interface Chemistry, Shandong University, Ministry of Education, Jinan 250100, PR China
[b] Department of Chemistry, University of Houston, Houston, TX, 77204, USA
[c] School of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, PR China
[d] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, PR China

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 26 July 2016
Accepted 28 July 2016
Available online 29 July 2016

Keywords:
Liquid crystals
Sensor
Cellulase
Cysteine

ABSTRACT

A liquid crystal (LC)-based sensor, which is capable of monitoring enzymatic activity at the aqueous/LC interface and detecting cellulase and cysteine (Cys), was herein reported. When functionalized with a surfactant, dodecyl β-D-glucopyranoside, the 4-cyano-4′-pentylbiphenyl (5CB) displays a dark-to-bright transition in the optical appearance for cellulase. We attribute this change to the orientational transition of LCs, as a result of enzymatic hydrolysis between cellulase and surfactant. Furthermore, by adding cellulase and Cu^{2+}, our surfactant-LCs system performs an interesting ability to detect Cys, even though Cys could not interact with surfactant or LC directly. Alternatively, through the strong binding between Cys and Cu^{2+}, cellulase was able to hydrolyze surfactant in the presence of Cu^{2+}, leading to the transition of LCs from dark to bright. The detection limit of the LC sensor was around 1×10^{-5} mg/mL and 82.5 μM for cellulase and Cys, respectively. The LC-based sensor may contribute to the development of low-cost, expedient, and label-free detection for cellulase and Cys and the design strategy may also provide a novel way for detecting multiple analytes.

1. Introduction

Thermotropic liquid crystals (LCs) possess demonstrated utility in the transduction of the stimulating events at the interface into amplified optical signals visible with the naked eyes [1–5]. Therefore, together with the designability and rapid responses to the target, LCs becomes a popular sensor design approach. The general mechanism of LC-based sensor results from molecular anisotropies and the unique liquid-crystalline phase, which cause the unique optical anisotropy, anchoring orientation, and elastic properties [6]. Recently, LC-based sensor has drawn great interest in the detection of biological analytes [7–11]. Compared to most current methods for monitoring biomolecules, the LC sensing approach could be used without laboratory-based analytical detectors, electric power, and molecular labels such as fluorophores or radioactive isotopes [12–14]. Furthermore, the aqueous/LC interface is especially sensitive to the bio-reaction due to the fluidity and the ease of molecular transport of the interface. Additionally, the interface could be further modified by adsorption of surfactant in a manipulative way for different systems [2,15]. Specifically, the efficient detection of cellulase and cysteine (Cys) could be achieved via the surfactant-decorated aqueous/LC interface due to its high sensitivity to the enzymatic reaction between cellulase and surfactant.

Cellulase is widely applied in paper industry, laundry detergent and textile [16,17] and plays an essential role in the carbon revolution of forest ecosystems [18,19]. Therefore, it is important to accurately monitor the activity of cellulase with high sensitivity and simplicity. Many well-established measurements such as the flow injection spectrophotometric method [20], agar-based method [21], and the fluorescence-based technique [22] have been developed, but they often required prolonged incubation, complex operation, sophisticated equipment and the involvement of labels. Cys, as an intracellular thiol, plays a critical role in some biological events including proteins synthesis, detoxification, and metabolism. A low level of Cys could result in growth retardation, hair depigmentation, edema, liver damage, weakness and skin lesions [23–25]. So Cys could help to provide useful significant information for the clinical diagnostics of various diseases, and its quick and reliable detection is very important. To date, several methods based on the interaction between thiol groups and metal ions with the colorimetric, luminescent and electrochemical sensing platform have been developed to determine the presence

* Corresponding author.
E-mail address: ylmlt@sdu.edu.cn (L. Yu).

http://dx.doi.org/10.1016/j.colsurfb.2016.07.059
0927-7765/

of Cys [26–28]. However, the existing methods often need laboratory measurements and tedious preparation jobs. Therefore, the new strategies to monitor Cys in a convenient way is still urgently needed, especially within the concentration range of 200–300 μM, which is the normal level in human body [27].

In this study, we constructed a simple and label-free sensing platform by confining the LCs in the gold grid to detect cellulase and Cys based on the enzymatic reaction between cellulase and surfactant (Fig. 1a). The dodecyl β-D-glucopyranoside monolayer formed at the aqueous/LC interface, and was hydrolyzed by cellulase. Then the enzymatic hydrolysis induced the reorientation of LCs from a homeotropic (Fig. 1b) to planar (Fig. 1c) state. And this process is corresponding to the transition of optical images from dark (Fig. 1d) to bright (Fig. 1e). Cu^{2+} could inactivate cellulase, but Cys could chelate with Cu^{2+} prior to prevent cellulase from inhibiting. After introducing the aqueous solution with Cys, cellulase and Cu^{2+}, the LC images maintained bright, which was different with the dark appearances of LCs in contact with aqueous solution of cellulase and Cu^{2+} only, so the Cys could be monitored accordingly. The method herein provides a strategy to design a sensitive and convenient sensing platform for cellulase and Cys in practical application.

2. Experimental

2.1. Materials

Cellulase, cysteine (Cys), and dodecyl β-D-glucopyranoside were purchased from Shanghai Shfeng Biological Technology Co., Ltd., China. Phosphate buffered saline (PBS) (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl; pH = 7.4) was obtained from Sigma-Aldrich. Nematic liquid crystal 4-cyano-4′-pentylbiphenyl (5CB), octadecyltrichlorosilane (OTS), heptane, *p*-hydroxybenzoic acid, L-arginine, L-alanine, L-lysine, glycine and glutamic acid were supplied by J&K Scientific Co., Ltd., China. Gold specimen grids (G75 with hole pitch of 340 μm, bar width of 55 μm, hole width of 285 μm) were purchased from GILDER, cleaned with ethanol and dried under a stream of nitrogen. Microscope glass slides were immersed into "piranha solution" for 30 min at 80 °C rinsed sequentially with water, ethanol and methanol, and dried under nitrogen, then followed by heating to 110 °C overnight. The "piranha-cleaned" glass slides were treated with OTS/heptane solution, and then washed with methylene chloride and dried under nitrogen [29].

2.2. Preparation of optical cells

The gold specimen grids were placed onto the surface of OTS-coated glass. Then, ~1 μL 5CB was distributed onto each grid, and the excess LCs was removed by a 20 μL capillary tube. Subsequently, 100 μL aqueous solution prepared in PBS was introduced onto the surface of 5CB to construct the interface of aqueous/LC. All the samples were measured at least three times.

2.3. Optical examination of LC textures

Images of the grid cells in the experiment were recorded by polarized light microscope (XPF-800C, Tianxing, Shanghai, China) under crossed polarizers with a digital camera (TK-9301EC, JVC, Japan). The optical appearances of 5CB were obtained by a 2.5 × objective lens at room temperature. The bright area coverage ratio (*B*r) of the optical image was acquired by utilizing Adobe Photoshop CS 5.

2.4. Assay for solution preparation

In the assay of cellulase inhibition, cellulase was pre-incubated with *p*-hydroxybenzoic acid or Cu^{2+} at 25 °C for 1 h, respectively. Before detecting Cys, the aqueous solutions containing cellulase (0.001 mg/mL), Cu^{2+} (0.01 mM) and Cys with different concentrations were incubated at 25 °C for 1 h. All aqueous solutions were prepared in PBS.

3. Results and discussion

3.1. Monitoring the activity of cellulase at dodecyl β-D-glucopyranoside-decorated aqueous/LC interface

Herein, we adopted 0.1 mM dodecyl β-D-glucopyranoside to decorate aqueous/LC interface. The optical appearances of LCs transferred from bright (Fig. 2a) to dark (Fig. 2b) immediately after introducing the surfactant solution into the optical cells. The bright-to-dark transition in the optical appearance of LCs corresponded to the planar-to-homeotropic orientation change of 5CB at the aqueous/LC interface. The perpendicular orientation of LCs was governed by the self-assembled monolayer of dodecyl β-D-glucopyranoside, which was triggered by the hydrophobic interaction between 5CB and surfactant [30]. In many cases, the stability of LC-based sensor within a certain period of time may critically influence its practical applications. In this work, the stability of the surfactant monolayer is a critical factor. Here the optical appearance was found to be able to maintain uniformly dark throughout the observation time (Fig. S1). Meanwhile, it has also been confirmed that the monolayer of 0.1 mM dodecyl β-D-glucopyranoside formed at the aqueous/LC interface was slightly affected by the concentrations of H^+ and salt in a wide range of concentrations in our previous study [31].

Then we measured the optical signal of LCs after 1 mg/mL cellulase was added onto the dodecyl β-D-glucopyranoside-decorated interface. The optical appearance of LCs turned bright from dark gradually, and became bright completely within 20 min (Fig. 2c–f). We speculate that the dark-to-bright shift in the optical appearance may be attributed to the enzymatic hydrolysis between dodecyl β-D-glucopyranoside and cellulase, and consequently the stability of surfactant monolayer is disturbed. In this study, the surfactant acted as not only the decorator of the LC sensor, but also the substrate for cellulase. Therefore, dodecyl β-D-glucopyranoside was considered as a direct regulator in orientational transition of 5CB.

3.2. Imaging sensitivity and specificity of cellulase at the dodecyl β-D-glucopyranoside-decorated aqueous/LC interface

After confirming the feasibility of our LC-based sensor for detecting cellulase, then we examined the sensitivity and specificity. To determine the detection limit of cellulase in this system, the time-dependent responses of LCs at different concentrations of cellulase were investigated. After transferring 0.1 mg/mL cellulase, the bright region gradually expanded from margin to the center, as shown in Fig. 3a. And then the entire square area was completely bright after 30 min. Longer time was required for the complete transition into bright appearance, when the concentrations of cellulase were lower. Furthermore, we calculated the bright area coverage ratio (*B*r) for different cellulase concentrations, and plotted them to their respective consumed times (Fig. 3b). *B*r of the optical images were obtained via calculating pixels of the bright area divided by pixels of the integral LC region using Adobe Photoshop CS 5. These results suggest that the limit of detection (LOD) for cellulase was around 0.001 mg/mL in buffer solution with our experimental operation.