J Mater Sci (2012) 47:969–975
DOI 10.1007/s10853-011-5876-y

Using liquid crystals for the real-time detection of urease at aqueous/liquid crystal interfaces

Qiong-Zheng Hu · Chang-Hyun Jang

Received: 20 June 2011 / Accepted: 10 August 2011 / Published online: 25 August 2011

Abstract In this study, we developed a simple and label-free liquid crystal (LC)-based biosensor for real-time screening of the presence of urease in aqueous solutions. Nematic liquid crystal, 4-cyano-4′-pentylbiphenyl (5CB), when treated with ultraviolet light, showed a bright to dark optical response to the emergence of ammonia in the aqueous phase, indicating a planar to homeotropic alignment of LC. The ammonia-driven optical signal could be ascribed to the orientational transition of 5CB, caused by the deprotonation of 4-cyano-4′-biphenylcarboxylic acid (CBA), which was the main product of UV-treatment and the screening of the electrostatic interactions at the aqueous/LC interface. Due to the high spatial resolution and fast response, this method was successfully used to detect urease belonging to the family of amidohydrolase, which hydrolyzes urea into ammonia and carbon dioxide. A urease concentration as low as 1 nM could be detected and monitored in real-time. Moreover, no signal was detected when a divalent copper ion, which blocks the active sites of urease, was used. This result further confirmed our initial hypothesis that orientational transitions of LC were induced from the enzymatic reaction. The results reported in this article suggest that our method may potentially have clinical utility for the specific detection of *Helicobacter pylori*. In addition, this ammonia-based LC biosensor may be used for the sensitive detection of other amidohydrolases.

Introduction

Pathogenic bacteria detection, which is considerably important to human safety and health, has been achieved through a variety of different methods including highly sensitive traditional techniques such as PCR, ELISA, and colony counts, and effective real-time detection methods involving optical, electrochemical, and piezoelectric instruments [1].

As a member of the most prevalent human pathogens [2], *Helicobacter pylori* (Hp), which is a crucial infectious agent of chronic diseases such as dyspepsia, gastritis, and peptic ulcers in the stomach and duodenum, is a causative agent of gastric carcinoma [3–6]. The International Agency for Research on Cancer (IARC) has ranked Hp as the No. 1 carcinogen in humans; thus, a large number of studies have been dedicated to the detection of this highly pathogenic microbe. The most important and remarkable characteristic of this bacteria is its abundant production of urease with high activity. Due to this property, urease tests are often used as an effective and efficient method to detect Hp infection in clinical samples [7, 8].

Urease, which is produced in large amounts by Hp, can hydrolyze available urea into two ammonia molecules and carbon dioxide. Monitoring changes in the pH of bulk solutions due to the urease reaction is the most widespread analytical method used to assess the presence of Hp in clinical samples. As ammonia is ionized into ammonium and hydroxide ions, the pH value of the aqueous solution increases [9].

Liquid crystalline materials can be used to amplify and transduce biomolecular events into optical outputs that are

Electronic supplementary material The online version of this article (doi:10.1007/s10853-011-5876-y) contains supplementary material, which is available to authorized users.

Q.-Z. Hu · C.-H. Jang (✉)
College of BioNano Technology, Kyungwon University,
San 65, Bokjeong-Dong, Sujeong-Gu, Seongnam,
Gyeonggi-Do 461-701, Korea
e-mail: chjang4u@kyungwon.ac.kr

visible by the naked eyes [10–13]. The orientational properties of liquid crystal (LC) enable LC-based sensors to be a simple, effective, and promising tool for detection with high spatial resolution [14, 15]. Compared to traditional analytical approaches, this method has a strong advantage because it does not require the use of labeled molecules [16], complex instrumentations [17], or laborious techniques [18].

In recent years, studies on LC-based biosensors have also been coupled to the use of biomimetic membranes made of synthetic polymers [19, 20], peptide amphiphiles [21, 22], proteins [23], or DNA [24] adsorbed at aqueous/LC interfaces. Previous studies have shown that ordering transition of LC can occur when amphiphilic polymers are assembled at planar aqueous/LC interface. External stimuli such as changes in the pH of the aqueous phase or the presence of oppositely charged polyelectrolytes can induce an optical response in the LC. In particular, Kinsinger et al. assembled polymers with pH sensitive groups at an aqueous/LC interface and observed optical responses that occurred from orientational transitions of the LC through a polarized microscope. These results demonstrated that the polymer reorganized due to reversible pH changes in the aqueous solution. The optical appearance of LC was significantly different when the pH was changed from 5.0 to 9.0 [25]. However, the response of this system was very slow (10 h), which has limited its widespread use.

Recently, Bi et al. developed an LC-based pH sensor by doping 4-cyano-4′-pentylbiphenyl (5CB) with 4′-pentylbiphenyl-4-carboxylic acid (PBA), which has a pH-sensitive functional group and a similar molecular structure to 5CB. In this literature, penicillinase was immobilized on metal bars of a copper grid, and the hollow square regions of the grid were filled with PBA-doped 5CB. Due to the release of hydronium ion (H^+) generated in the enzymatic reaction, changes in the optical appearance of the LC from dark to bright were observed after introducing penicillin G in PBA (pH = 7.0) into the optical well [26]. Using this approach, they could detect a small amount of H^+ released from enzymatic reactions in a solution with high buffer capacity. However, the penicillinase that was immobilized on the copper grids was usually inhomogenous and had a low spatial resolution, which limits the use of this system as a general tool for studying enzymatic reactions.

Park et al. reported that when nematic 5CB was exposed to ultraviolet (UV) light, a range of reaction products were generated, where 4-cyano-4′-biphenylcarboxylic acid (CBA) was the main product of the photochemical degradation. They found that photochemical treatment enhanced the orientational property of 5CB on solid surfaces containing functional groups, thereby increasing the optical contrast in the LC images [27]. Since CBA has a similar molecular structure and properties as PBA, the CBA-doped 5CB could also be used as a material in the construction of LC-based pH sensors at the aqueous/LC interface.

In this study, we investigate the orientational properties of UV-treated 5CB at aqueous/LC interfaces, and demonstrate the feasibility of using this material to monitor the presence of urease in the aqueous phase. When the UV-tailored 5CB was in contact with the mixture of urease and urea in aqueous solution, an optical change of the liquid crystal from bright to dark was observed through crossed polarizers, indicating an orientational change of the 5CB from a planar to homeotropic state. We also examined the effect of divalent copper ions, which inhibit this enzyme-catalyzed reaction, to further confirm our initial hypothesis. This type of LC-based sensor may be employed as a tool for the selective and sensitive detection of pathogens and enzymes in real-time.

Experimental details

Materials

Nematic liquid crystal 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 meshes, pitch 508 μm, hole 425 μm, bar 83 μm) were purchased from GILDER. Octyltrichlorosilane (OTS), methanol, methylene chloride, sulfuric acid, hydrogen peroxide (30% w/v), urease, urea, sodium hydroxide, and copper nitrate hydrate were purchased from Sigma-Aldrich. All aqueous solutions were prepared with deionized water (18 MΩ cm) of high purity, using a Milli-Q water purification system (Millipore, Bedford, MA).

Photochemical degradation of 5CB using UV light

UV-treated 5CB was prepared as described previously [27]. In brief, 0.6 g of 5CB in an 8 mL beaker was placed under a Spectroline EN-280L longwave UV lamp (365 nm) that was equipped with two 8 W tubes and a filter assembly. The distance between the 5CB and the lamp was about 8 cm, and the entire system was placed in a box to block external light. The samples were exposed to the UV light for 30 and 48 h, respectively. Based on a previous study, the concentration of CBA in the final sample (48 h exposure) was about 0.8%.

Preparation of aqueous solutions for urease test

Aqueous mixtures of urea and urease were prepared by directly integrating the aqueous solutions of the two components. In the inhibition experiments, a mixture of urease

and copper nitrate hydrate were first incubated for 45 min, and then the urea solution was added into the mixture. These solutions were maintained at room temperature (~25 °C) for predetermined time intervals.

Cleaning of substrates

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 1 h at 80 °C. After removal from the cleaning solution, the substrates were rinsed with copious amounts of deionized water, ethanol, and methanol and dried under a stream of gaseous N_2. The cleaned substrates were stored overnight in an oven at 120 °C.

Preparation of OTS-treated glass slides

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. The OTS slides were tested for homeotropic alignment by observing the orientation of 5CB sandwiched between two OTS slides. Any slide that did not display homeotropic alignment was discarded.

Preparation of optical cells

The OTS-coated glass slides were fixed at the bottom of an eight-well chamber slide. Copper specimen grids were then placed onto the OTS-coated glass slide. 5CB of 2.0 μL was dispensed into each grid, and the optical cell was heated to 50 °C (above the nematic-isotropic transition temperature of 5CB, ~35 °C). Excess LC was removed by placing a 20 μL capillary tube in contact with the 5CB droplet on the grid. Subsequently, 500 μL of the aqueous solution of interest was immersed into the optical cell at room temperature.

Optical examination of LC textures

A polarized light microscope (ECLIPSE LV100POL, Nikon, Tokyo, Japan) was used to image the optical textures formed by light transmitted through the optical cells that were filled with nematic 5CB. All images were obtained using a 4× objective lens between crossed polarizers. Images of the optical appearance of each liquid crystal cell were captured with 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. Images were quantified by interpreting them to a gray scale of intensities, and their luminosities were analyzed using image processing software (Adobe Photoshop) [28].

Results and discussion

Orientational behavior of photochemically degraded 5CB at aqueous/LC interfaces

Past studies have shown that nematic 5CB can be used to detect biomolecular events at fluid interfaces [14, 15, 19–27, 29]. When water-immiscible LC is deposited into the holes of a transmission electron microscopy (TEM) grid supported on a glass surface treated with OTS, a stable and planar interface is obtained, because the grid can prevent the hydrophobic 5CB from dewetting. When the 5CB-filled TEM grid is immersed into the aqueous solution of interest, it produces a different optical appearance through a polarized microscope, which reflects the orientation of the LC.

The goal of this study was to use photochemically degraded 5CB for the detection of enzyme-catalyzed reactions. The reasons for using this material to develop the detection of enzyme-catalyzed reactions are as follows. (a) The orientational properties of UV-treated 5CB at chemically functionalized surfaces have been studied and were shown to be potentially useful for the development of LC-based diagnostic tools. However, the mechanism for the orientational transition of UV-treated 5CB at aqueous/LC interface is still unclear, which limits further application of the system. (b) The major product of UV-treated 5CB is CBA, which has a structure similar to 5CB. Since it contains a carboxylic acid terminus, CBA should be sensitive to pH and electrolyte concentration in the aqueous phase. In addition, due to its amphiphilic property, the deprotonated CBA (CBA^-) may self-assemble at the aqueous/LC interface, which is similar to the behavior of surfactants or lipids at an aqueous/hydrophobic interface. It is known that the addition of electrolytes to an aqueous solution can promote the adsorption of ionic surfactants onto the aqueous/hydrophobic interface due to the ability of electrolytes to screen the electrostatic repulsion between the head groups of ionic surfactants. Previous studies have shown that the interaction between the aliphatic chains of lipids and LCs can cause the homeotropic orientation of 5CB at the aqueous/LC interface [29]. Therefore, we hypothesize that when UV-treated 5CB is exposed to an aqueous solution of ammonia, which could be ionized into hydroxide and ammonium ion, an orientational transition of 5CB from planar (Fig. 1a) to homeotropic (Fig. 1b)

alignment will occur, and therefore the optical appearance of the LC will change from bright to dark.

When UV-treated 5CB (30 h) that had been confined to a copper grid was immersed into DI water, the liquid crystal had a bright and colorful appearance (Fig. 1c), indicating a planar or tilt alignment of 5CB. We replaced the DI water in the optical cell with 0.5 M aqueous ammonia. The optical appearance changed from bright to dark (Fig. 1d) within 5 min, which is indicative of an orientational transition of the LC from planar to homeotropic state. However, when pure 5CB (without UV treatment) was incubated with aqueous ammonia, the pure 5CB remained bright and colorful (data not shown), suggesting no ordering transition occurred in the LC. Based on this result, we concluded that it would be possible to use UV-tailored 5CB in the development of a simple method for the detection of ammonia.

We presumed that the combined effect of hydroxide and ammonium ions, which are produced by the hydrolysis of ammonia, would induce an orientational transition of LC. Because the acid moiety of CBA is sensitive to pH, an increase in the hydroxide ion concentration in the aqueous phase facilitates the deprotonation of CBA at the interface. In addition, ammonium ions can screen the repulsive interaction between the carboxylate head groups, and therefore increase the areal density of CBA at the aqueous/LC interface.

To test the hypothesis that the combined action of hydroxide and ammonium ions can induce an orientational transition of UV-treated 5CB, we examined the effect of pH and salt on the orientational behavior of 5CB at the aqueous/LC interface. When we immersed the TEM grid filled with UV-treated 5CB into a 0.5 M aqueous sodium chloride at pH 11, the optical appearance immediately changed from bright to dark (Fig. S1a). However, when UV-treated 5CB was incubated with only basic water (pH = 11) or with 0.5 M aqueous sodium chloride, the 5CB remained bright and colorful (Fig. S1b, c), indicating that no ordering transitions took place. The same set of experiments was also conducted using pure 5CB without UV treatment (Fig. S1d–f) and no change in optical appearance was observed for pure 5CB. This result indicates that the action of deprotonated CBA induced an orientational transition of LC at the aqueous/LC interface.

Real-time detection of the urease-catalyzed reaction at the aqueous/LC interface

Based on the above results, we predicted that the orientational transition of UV-treated 5CB could be observed through crossed polarizers when a mixed solution of urease and urea was introduced into the optical cell. Since urease can hydrolyze urea into ammonia and carbon dioxide, the released ammonia would be ionized into hydroxide and ammonium ions in an aqueous solution. The hydroxide ions could then induce the deprotonation of CBA and the ammonium ions could screen the repulsive interaction between the carboxylic head groups. The combination of these two procedures would facilitate the adsorption of CBA at the aqueous/LC interface, which would induce an orientational transition of LC from a planar to homeotropic state.

An optical cell was prepared with a UV-treated 5CB-filled TEM grid supported on a glass surface that was treated with OTS. After introduction of DI water, the 5CB appeared bright and colorful (Fig. 2a), which occurred due

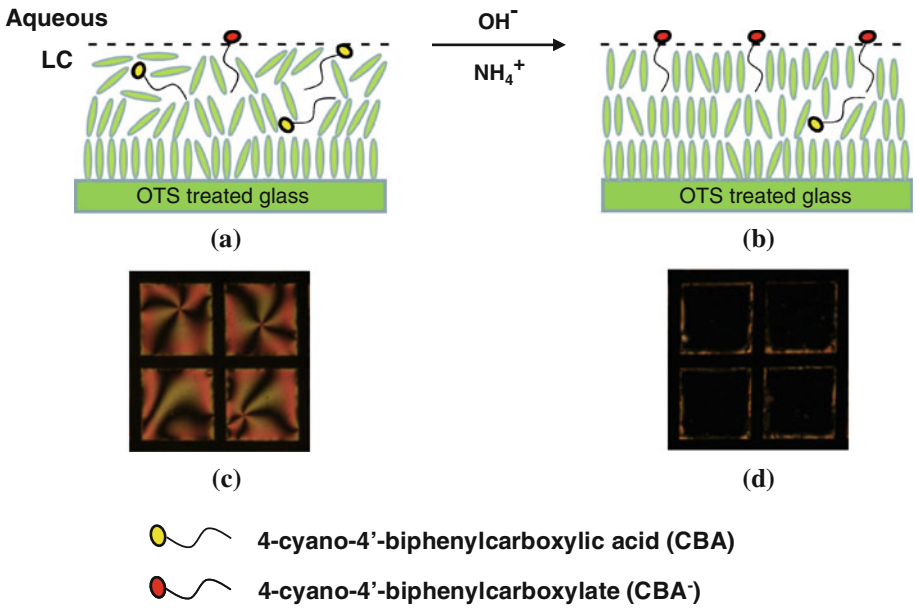

Fig. 1 Schematic illustration of the orientational transition of photochemically degraded 5CB from **a** planar alignment to **b** homeotropic alignment. Polarized light microscope images of photochemically degraded 5CB (30 h) in contact with **c** DI water and **d** 0.5 M ammonia. The UV-treated 5CB was hosted in a copper grid (pitch 508 μm, hole 425 μm, bar 83 μm)

to the in-plane birefringence that is associated with a planar or tilt orientation of the LC at the interface. The DI water was then replaced with a mixture of 100 nM urease and 0.5 M urea, which was pre-incubated at room temperature for 30 min. Then, the optical appearance of 5CB changed swiftly from bright to completely dark (Fig. 2d) within less than 1 min, indicating the orientation of LC changed from a planar to perpendicular state.

To further verify that the urease and urea mixture was responsible for the orientational transition of LC, we conducted two control experiments: incubation of the optical cell with only 100 nM urease or 0.5 M urea solution. Figure 2b and c shows the optical appearance of the UV-treated 5CB when immersed in a 100 nM urease or 0.5 M urea solution, respectively. In both cases, the optical appearance of the UV-treated 5CB cell remained bright and colorful for more than 2 h. Therefore, we concluded that the combined action of ammonium and hydroxide ions generated from the enzyme reaction was responsible for the orientational transition of the LC from a planar to homeotropic state. The above results demonstrate that UV-treated 5CB can be used to specifically amplify and transduce the urease reaction into an optical signal.

Time-dependent interfacial phenomenon

In order to more clearly understand the mechanism for the 5CB transition due to the enzyme reaction, the change in the optical appearance was observed over a longer period of time. A mixture of 10 nM urease and 0.5 M urea was incubated for 30 min before transferring the solution into the optical cell. 5 min after contacting 5CB with the reaction mixture, the luminosity gradually decreased (Fig. 3a–c), which indicates a gradual change in the orientation of LC from tilt to homeotropic state at the aqueous/LC interface. However, the luminosity gradually increased after a longer incubation period (Fig. 3d–f), implying a homeotropic to tilt shift in the orientation of LC. Characteristic time-dependent changes in the optical appearance of LC were shown in Fig. S2.

Previous studies have demonstrated that surfactants such as sodium dodecyl sulfate (SDS) induced changes in the optical appearances of LC from bright to dark when the concentration of aqueous SDS exceeded a critical value. The orientational transition of 5CB from planar-to homeotropic alignment was attributed to the interactions between 5CB and SDS molecules and it was shown that a sufficient areal density of SDS was needed at the aqueous/LC interface for this transition to occur. However, the optical appearance of 5CB returned to a colorful state when the surfactant-decorated interface was exposed to an aqueous phase with a lower SDS concentration. This transition occurred because of the desorption of SDS molecules from the interface to the aqueous phase, which resulted in a decrease in the areal density of SDS at the aqueous/LC interface [29].

Based on the results from SDS study, we concluded that the return of the optical transition of 5CB that had been in contact with the urease–urea mixture resulted from the competition between the deprotonation of CBA and the desorption of the deprotonated CBA from the aqueous/LC interface. As the deprotonation of CBA proceeded, the areal density of the self-assembled CBA^- increased at the interface. When it exceeded a critical value, the alignment of LC was perturbed and it changed from a planar to homeotropic state, resulting in a uniformly dark optical image. However, when the rate of deprotonation slowed down, the desorption of accumulated CBA^-, which is due to the diffusion of CBA^- into the aqueous phase from the interface, would dominate the orientational behavior of LC. The deprotonation rate could not sufficiently compensate of the CBA^- that had diffused out from the interface; thus, the orientational of LC reverted to a planar state.

Determination of detection limit

We then examined the detection limit of our system for urease. The sensitivity of this system is related to the concentration of urease and the incubation time of the urease–urea mixture before it was introduced into the

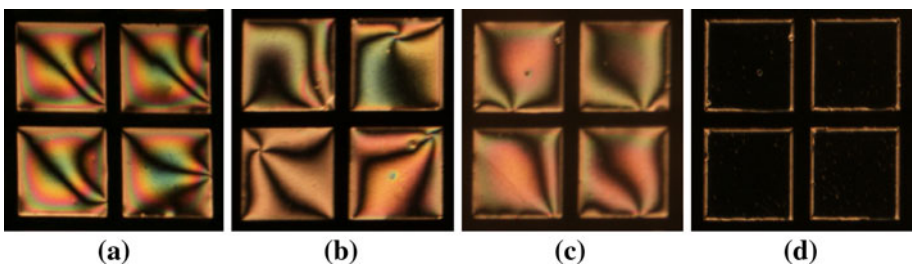

Fig. 2 Optical images of the photochemical degraded 5CB (48 h) viewed through a polarized light microscope when in contact with **a** DI water, **b** 100 nM urease, **c** 0.5 M urea, and **d** the mixture of 100 nM urease and 0.5 M urea. The UV-treated 5CB was hosted in a copper grid (pitch 508 μm, hole 425 μm, bar 83 μm)

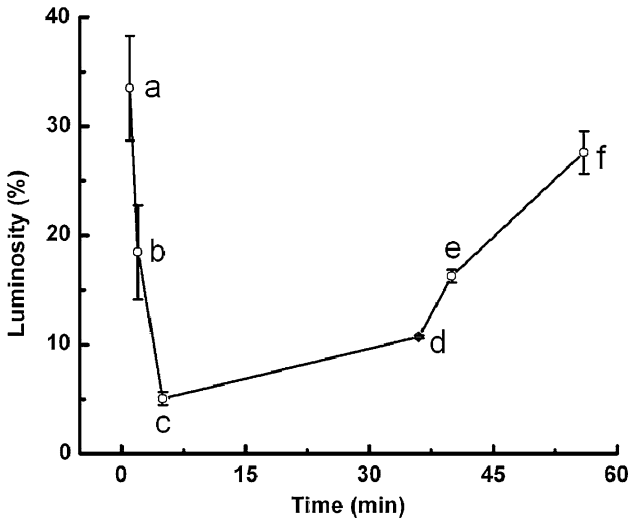

Fig. 3 Light intensity of the optical images as the photochemical degraded 5CB (48 h) was incubated with the mixture of 10 nM urease and 0.5 M urea for *a* 1 min, *b* 2 min, *c* 5 min, *d* 36 min, *e* 40 min, and *f* 56 min

aqueous/LC interface. The optical appearance of the UV-treated 5CB was examined when in contact with mixtures that contained different urease concentrations and that had been incubated for different time periods before being placed in contact with the mixed solution. A mixture of 50 nM urease and 0.5 M urea, which had been pre-incubated for 3 min, was first introduced into the optical cell. The LC immediately changed from a colorful to dark state (Fig. 4a), which represented an ordering transition of LC. When a mixture of 10 nM urease and 0.5 M urea was pre-incubated for 3 min and introduced into the optical cell, the 5CB had a gray to yellow optical appearance (Fig. 4b left) through the crossed polarizers, which indicates a nearly homeotropic orientation of 5CB at the interface. When the pre-incubation time was increased to 30 min, the cell appeared dark within 5 min (Fig. 4b right). The optical cell was then placed in contact with a mixture of 1 nM urease and 0.5 M urea that had been pre-incubated for different time periods (Fig. 4c). Almost no obvious change in the optical appearance was observed when the pre-incubation time was 3 min. The interference color changed from red to green when the mixture was incubated for 30 min, indicating a decrease in the tilt angle (relative to the surface normal) of the LC at the aqueous/LC interface. When the pre-incubation time was 60 min, the LC appeared almost black, suggesting the 5CB turned to a nearly homeotropic state. When the pre-incubation time was 90 min, a uniformly black image was observed within 5 min, indicating a homeotropic alignment of LC.

Based on the data above, we concluded that the reasonable detection limit of this urease sensor was around 1 nM. However, the detection limit could be even lower if the pre-incubation time of the enzyme–substrate mixture is extended.

The effect of inhibitor on the enzyme reaction

In order to further test the hypothesis that the orientational transition of CBA-doped 5CB was caused by the enzymatic hydrolysis of urea, we examined the effect of copper nitrate hydrate, which blocks the active sites of urease, on the LC

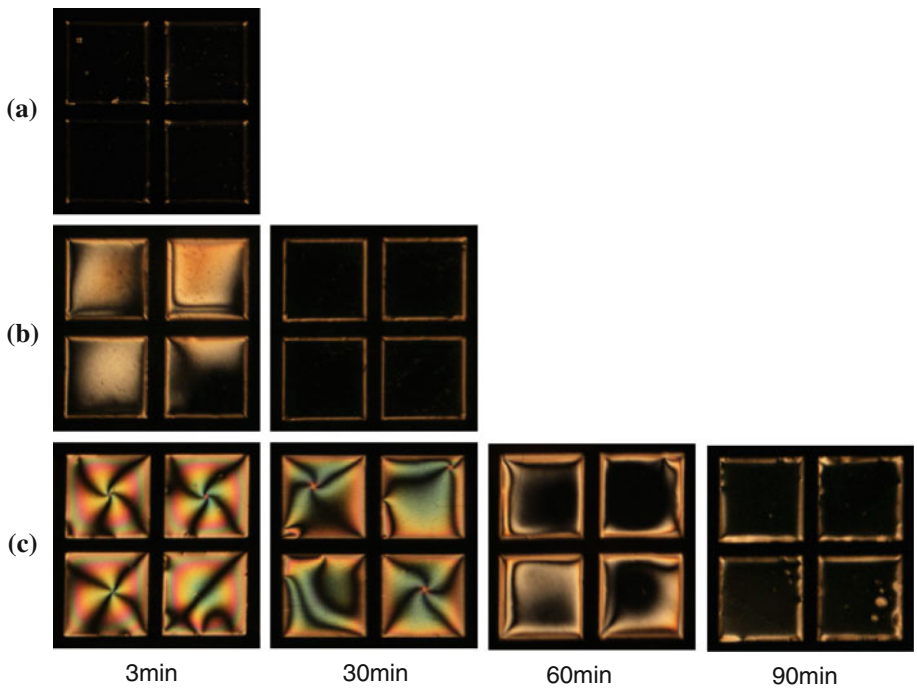

Fig. 4 Optical images of photochemical degraded 5CB (48 h) viewed through a polarized light microscope when in contact with a mixture of **a** 50 nM urease and 0.5 M urea, **b** 10 nM urease and 0.5 M urea, and **c** 1 nM urease and 0.5 M urea. The bottom numbers indicate the time the urease–urea mixture was incubated before it was introduced into the optical cell

transitions. It is known that the divalent copper ions inhibit this enzyme through an interaction between the metal ion and the thiol groups of the enzyme molecule, and the coordination of the copper ion to nitrogen- and possibly oxygen-containing functional groups in urease [9, 30, 31].

A mixed solution containing 200 nM copper nitrate hydrate and 20 nM urease was first made with a 10:1 molar ratio of inhibitor to enzyme. This mixture was then incubated for 45 min at room temperature. A 1 M urea solution, the volume of which was the same as the enzyme solution, was added into the enzyme–inhibitor mixture. The final concentrations of urease, urea, and inhibitor were 10 nM, 0.5 M, and 100 nM, respectively. Under these conditions, no obvious optical transition of LC was observed up to 90 min (Fig. S3). This result further confirms our initial hypothesis that the orientational transition of LC resulted from the enzyme catalysis reaction.

Conclusion

In this study, we developed a simple method that allows for the detection of urease in an aqueous solution using UV-treated 5CB which contains CBA. Due to the production of ammonia from the enzymatic reaction, the orientation of LC undergoes a planar to homeotropic transition, which can be easily visualized by the naked eye. The deprotonation of CBA induced by basic pH and the screening of the electrostatic repulsion between the carboxylic head groups by the electrolytes are two main reasons for the orientational transition of LC at the aqueous/LC interface. We confirmed that the orientational transition of LC occurred due to the urease catalysis reaction by investigating the effect of copper nitrate hydrate, which is an inhibitor of urease, on the LC transition. The concentration-dependent experiments showed that the detection limit of this system for urease was around 1 nM. These results suggest that the aqueous/UV-treated 5CB interface may be useful for the development of a label-free detection method for enzymatic reactions that can be imaged by LC.

Acknowledgement This study was supported by the Kyungwon University Research Fund (KWU-2011-R214).

References

1. Lazcka O, Campo F, Munoz FX (2007) Biosens Bioelectron 22:1205
2. Bardhan PK (1997) Clin Infect Dis 25:938
3. Laine L, Schoenfeld P, Fennerty MB (2001) Ann Intern Med 134:361
4. Schenk B, Kuipers E, Nelis G, Bloemena E, Thijs J, Snel P, Luckers A, Klinkenberg-Knol E, Festen H, Viergever P, Lindeman J, Meuwissen S (2000) Gut 46:615
5. Graham DY (1993) N Engl J Med 328:349
6. Bjorkholm B, Befrits R, Jaup B, Engstrand L (1998) J Clin Microbiol 36:3689
7. Williams CL (1997) J Infect 34:1
8. Mobley H, Hu L, Foxall (1991) Scand J Gastroenterol 26(Suppl): 39
9. Kuswandi B (2003) Anal Bioanal Chem 376:1104
10. Gupta VK, Skaife JJ, Dubrovsky TB, Abbott NL (1998) Science 279:2077
11. Jang CH, Tingey ML, Korpi NL, Wiepz GJ, Schiller JH, Bertics PJ, Abbott NL (2005) J Am Chem Soc 127:8912
12. Jang CH, Cheng LL, Olsen CW, Abbott NL (2006) Nano Lett 6:1053
13. Bi X, Huang S, Hartono D, Yang KL (2007) Sens Actuators B 127:406
14. Brake JM, Daschner MK, Luk YY, Abbott NL (2003) Science 302:2094
15. Brake JM, Abbott NL (2007) Langmuir 23:8497
16. Zako T, Nagata H, Terada N, Sakono M, Soga K, Maeda M (2008) J Mater Sci 43:5325. doi:10.1007/s10853-008-2776-x
17. Tourillon G, Dreesen L, Volcke C, Sartenaer Y, Thiry PA, Peremans A (2009) J Mater Sci 44:6805. doi:10.1007/s10853-009-3572-y
18. Park CO, Akbar SA, Weppner W (2003) J Mater Sci 38:4639. doi:10.1023/A:1027454414224
19. Kinsinger MI, Buck ME, Meli MV, Abbott NL, Lynn DM (2010) J Colloid Interface Sci 341:124
20. Lin IH, Meli MV, Abbott NL (2009) J Colloid Interface Sci 336:90
21. Park JS, Teren S, Tepp WH, Beebe DJ, Johnson EA, Abbott NL (2006) Chem Mater 18:6147
22. Park JS, Abbott NL (2008) Adv Mater 20:1185
23. Hartono D, Xue CY, Yang KL, Lanry Yung LY (2009) Adv Funct Mater 19:3574
24. Price AD, Schwartz DK (2008) J Am Chem Soc 130:8188
25. Kinsinger MI, Sun B, Abbott NL, Lynn DM (2007) Adv Mater 19:4208
26. Bi X, Hartono D, Yang KL (2009) Adv Funct Mater 19:3760
27. Park JS, Jang CH, Tingey ML, Lowe AM, Abbott NL (2006) J Colloid Interface Sci 304:459
28. Zhao Y-B, Yu J-H, Zhao H-F, Tong C-Y, Wang P-H (2011) Sens Actuators B Chem 155:351
29. Brake JM, Abbott NL (2002) Langmuir 18:6101
30. Saboury AA, Poorakbar-Esfahani E, Rezaei-Behbehani G (2010) J Sci Islam Repub Iran 21:15
31. Borisov SM, Wolfbeis OS (2008) Chem Rev 108:423