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Detection of catalase activity with aldehyde-doped liquid crystals confined in microcapillaries

Jinseob Rim, Chang-Hyun Jang*

Department of Chemistry, Gachon University, Seongnam-daero 1342, Sujeong-gu, Seongnam-si, Gyeonggi-do, 13120, South Korea

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ABSTRACT

In this study, a simple, rapid, and label-free sensor was developed for detecting the enzymatic activity of catalase (CAT) with liquid crystals (LCs) confined in microcapillaries. Inside a microcapillary functionalized with *n*-octyltrichlorosilane, aldehyde-doped LCs anchored radially so that a pattern of straight lines was observed under a polarized optical microscope (POM). However, once hydrogen peroxide (HP) oxidized the aldehyde into carboxylic acid, which has surface activity, the orientation of the LCs at the interface changed, resulting in a distinct pattern change, from straight to crossed. In this system, the enzymatic activity of CAT could be detected as it inhibits the oxidation by decomposing HP; as a result, the pattern changed back to the straight one. From the orientational and optical shift, the enzymatic activity of CAT was detected up to a concentration of 0.8 fM under mild experimental conditions and 8 aM at pH 9.0. This result suggests the need for further study of microcapillary systems to develop simple and sensitive sensors for biochemical interactions.

Introduction

Hydrogen peroxide (HP) is found in many kinds of aerobic organisms as a by-product of cellular respiration [1]. As one of the peroxide compounds which have strong oxidizing activity, HP can damage cells and their organelles, especially mitochondria, via oxidative stress [1]. However, despite its toxicity, HP is used in several processes, such as cellular defense [2,3], regulation [4,5], and signaling [5]. Studies have detected and tracked HP level directly through spectrometry [6,7], chemiluminescence [8,9], titrimetry [10], and electrochemistry [11,12], to investigate these cellular processes. Because most of those methods utilize enzymes to trigger a signal from HP, their limitations are based on the instability of protein immobilization and protein denaturation.

An alternative way to track the production of HP in biological environments is to detect catalase (CAT), which is produced by aerobic organisms to reduce the concentration of HP. CAT tends to accumulate greatly where HP is generated, because it decomposes HP into water and oxygen. Moreover, detecting CAT has clinical significance also, as some diseases, such as acatalasia, are related to CAT. Researchers have detected CAT using various techniques: spectrophotometry [13], colorimetry [14], surface plasmon resonance [15], and high-performance liquid chromatography [16]. However, the lowest limit of detection (LOD) for these approaches was at the picomolar level, and expensive apparatus were used, which limits their broad application.

Liquid crystal (LC)-based sensors have peaked interest in the biosensing arena because of their several advantages over other sensors [17–22]. They work in an ambient environment, meaning they require neither high temperature nor high pressure. Above all, they magnify bio/chemical events at the nanoscale, so the events can be visualized with a polarized optical microscope (POM) without any label on the analytes. In practice, they are employed in several platforms, such as liquid-liquid interfaces [20], oil-in-water emulsions [23], and droplet patterns [24] with nematic LCs, or cholesteric LCs [21,22]. In our latest study, our group detected the enzymatic activity of CAT up to the femtomolar level with 2 h preincubation under optimal conditions [24].

In the current study, we aimed at developing a novel sensing platform, wherein LC droplets in a glass microcapillary would exhibit distinct optical appearances under POM in response to the target reaction [25,26]. Because it constitutes nearly closed system, this platform is so stable that the optical signal could remain for 120 h [26]. We hypothesized that, if an aldehyde-doped LC droplet meets the HP-containing solution inside the microcapillary, oxidized aldehyde molecules would help the LC molecules anchor homeotropically to the LC/aqueous interface and, consequently, the optical cell would exhibit a characteristic pattern under POM. Otherwise, if CAT decomposes HP in the solution, LC molecules would anchor radially along the axis because unoxidized aldehyde molecules do not have any effect on the

* Corresponding author.
E-mail address: chjang4u@gachon.ac.kr (C.-H. Jang).

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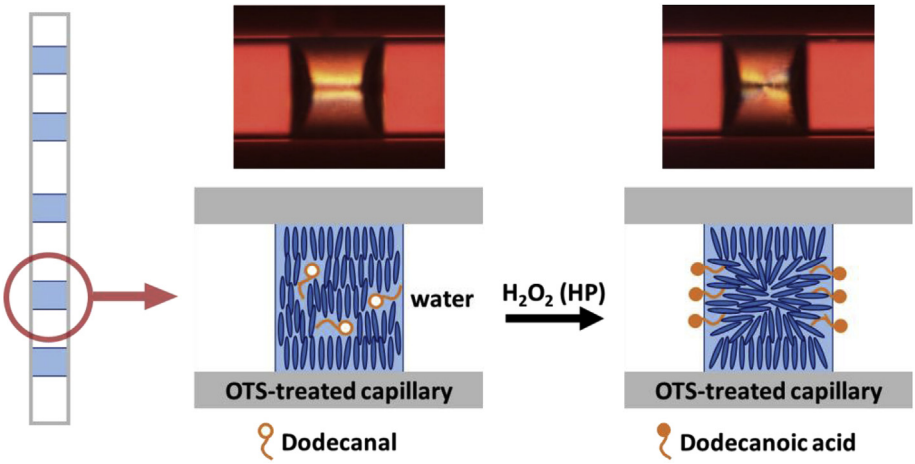

Fig. 1. Schematic illustration of an LC droplet inside an OTS-treated microcapillary, in which the orientation of 5 C B at the interface was changed by oxidation of dodecanal.

orientation of LC molecules, resulting in another pattern under POM (Fig. 1). We continued the study using these distinct optical patterns to determine whether the reaction occurred or not.

Experimental details

Materials

4-Cyano-4′-pentylbiphenyl (5 C B) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Hirschmann micropipettes (for 1–5 μL, inner diameter 580 μm; microcapillaries), dodecanal, dodecanoic acid, catalase from bovine liver (CAT), phosphate buffered saline (PBS), trypsin from bovine pancreas, lysozyme from chicken egg white, and urease from *Canavalia ensiformis* were purchased from Sigma Aldrich (St. Louis, MO, USA). *n*-Octyltrichlorosilane (OTS) was purchased from Alfa Aesar (Ward Hill, MA, USA). *n*-Heptane, sulfuric acid, and HP were obtained from Daejung Chemicals & Metals Co., Ltd. (Gyeonggi-do, South Korea). Deionized (DI) water (18 MΩ/cm) from Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all aqueous solutions.

Functionalization of microcapillaries with OTS

The microcapillaries were cleaned using a “piranha solution” (70% H_2SO_4/30% H_2O_2; Caution: This “piranha solution” reacts violently with organic materials and should be handled with extreme caution; do not store this solution in closed containers) for 30 min at 80 °C. After removal from the piranha solution, the microcapillaries were sequentially rinsed with abundant deionized (DI) water, ethanol, and methanol; dried with a stream of gaseous N_2, and stored in an oven overnight at 120 °C.

The piranha-cleaned microcapillaries were immersed in an OTS/*n*-heptane solution (1 mM) for 30 min, and then rinsed with methylene chloride and dried under a stream of N_2.

Preparation of CAT solution and reacting solution

CAT was first dissolved in 50 mM PBS at a concentration of 1 mg/mL as a stock solution, following the manufacturer's instruction. Then, the CAT solution and HP-containing target solution were mixed for a final buffer concentration of 0.1 mM and incubated for 1 h at room temperature. The mixture was used in the experiments immediately thereafter.

Visualization of orientational behavior of LCs

Using capillary force, 1 μL of dodecanal-doped 5 C B was dispensed into the microcapillary. Approximately 3.5 μL of the mixture of interest was injected with a syringe. The mixture-loaded microcapillary was placed in a polarization light microscope (POM; ECLIPSE LV100POL, Nikon, Tokyo, Japan) to visualize the optical appearance of nematic 5 C B inside the microcapillary. All images were acquired using a 4 × objective lens under crossed polarizers. Each optical texture was captured with a digital camera (DS-2Mv, Nikon, Tokyo, Japan) attached to the POM at a resolution of 1600 × 1200 pixels, a gain of 1.00 ×, and a shutter speed of 1/30 s. The vertical dimension of each droplet was 400 μm. The room temperature was kept above 23 °C which is in the nematic range (22.5–35 °C) of 5 C B [27].

Results and discussions

Orientational behavior of aldehyde-doped 5 C B at the interface

We initially examined the optical appearance of 5 C B confined in the microcapillaries for each condition. As previous studies demonstrated, pure 5 C B confined in OTS-treated microcapillary anchors radially, inducing the image of two bright lines under POM (Fig. 1) [25,26]. When dodecanal was doped into 5 C B over a 1 v/v% level, the optical appearance of the LC droplet became a 4-Pl pattern after contacting 0.1 mM PBS (Fig. 2A). This indicated that the orientation of 5 C B at the interface changed from planar to homeotropic (Fig. 1). This result was undesirable, because this kind of change should appear only after HP oxidizes dodecanal into dodecanoic acid. Assuming dodecanal molecules interacting with salt ions at the interface affected the orientation of the 5 C B, we reduced the concentration of dodecanal in 5 C B. Below 0.8 v/v%, the pattern was consistently straight, implying that the number of dodecanal molecules that interacted with salt ions was insufficient to change the orientation of 5 C B (Fig. 2B). However, if the concentration of dodecanal was too low, the orientation of 5 C B might not change even after all the dodecanal molecules oxidized into dodecanoic acid. Thus, we doped dodecanoic acid instead of dodecanal to confirm how many dodecanal should be oxidized to change the orientation of 5 C B at the interface. Above the concentration of dodecanoic acid corresponded to the molar concentration of 0.5 v/v% dodecanal, the orientation of 5 C B changed from planar to homeotropic at the interface, appeared in 4-Pl pattern (Fig. 2C). On the other hand, below “0.4%” of dodecanoic acid, the optical appearance was two-straight lines, indicating that the orientation of 5 C B remained planar at the interface (Fig. 2D). From these results, we concluded that at least