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A cationic surfactant-decorated liquid crystal sensing platform for simple and sensitive detection of acetylcholinesterase and its inhibitor

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ABSTRACT

In this paper, construction of the liquid crystal (LC)-based sensing platform for simple and sensitive detection of acetylcholinesterase (AChE) and its inhibitor using a cationic surfactant-decorated LC interface was demonstrated. A change of the optical images of LCs from bright to dark appearance was observed when the cationic surfactant, myristoylcholine chloride (Myr), was transferred onto the aqueous/LC interface, due to the formation of a stable surfactant monolayer at the interface. A dark-to-bright change of the optical appearance was then observed when AChE was transferred onto the Myr-decorated LC interface. The sensitivity of this new type of LC-based sensor is 3 orders of magnitude higher in the serum albumin solution than that only in the buffer solution. Noteworthy is that the AChE LC sensor shows a very high sensitivity for the detection of the enzyme inhibitor, which is around 1 fM. The constructed low-cost LC-based sensor is quite simple and convenient, showing high promise for label-free detection of AChE and its inhibitors.

1. Introduction

Acetylcholinesterase (AChE) is a high-efficiency enzyme for the hydrolysis of neurotransmitter acetylcholine (ACh) that plays a key role in regulation of the neutral response system (Duford et al., 2013; Huynh et al., 2013; Liu et al., 2013; Pimsen et al., 2014; Zhao et al., 2015). For example, Alzheimer's dementia (AD) is a common disease for elderly people that is related to a low level of ACh (Zhou et al., 2013). Rational pharmaceutical treatment of AD with AChE inhibitors is clinically applied to increase the concentration of ACh. However, excess of acetylcholine causes neuromuscular paralysis and is lethal to human (Li et al., 2011). Therefore, it is significantly important to detect AChE and its inhibitors. Although many well-established techniques, including Ellman's method (Ellman et al., 1961), colorimetric assay (Li et al., 2011; Luckham and Brennan, 2009), fluorescent approaches (Saa et al., 2010; Wang et al., 2009; Zhang et al., 2013), and various electrochemical methods (Miao et al., 2010; Zhao et al., 2015) have been extensively applied, they encounter drawbacks such as low detection sensitivity, long measuring time, labor-intensive operation, complex instrumentation, and use of molecular labels or nanoparticle probes. Thus it is particularly worthy of constructing new type of sensing devices for rapid and label-free screening of AChE and its inhibitors in a simple and convenient way with high sensitivity.

Liquid crystals (LCs) have drawn great interest for sensitive amplification and transduction of chemical and biological events into optical signals that are visible by the naked eye (Brake et al., 2003, 2005; Hussain et al., 2014; Khanab and Park, 2012; Lin et al., 2013; Popov et al., 2014; Sen et al., 2013). Due to unique liquid-crystalline phase properties, LCs enable construction of a miniaturized sensing device without the need of synthetic macromolecules, molecular labels and electric power (Birchall et al., 2008; Hartono et al., 2009; Hussain et al., 2009). High sensitive and selective LC-based sensors have been reported for monitoring varieties of biomolecules (Bera et al., 2014; Chang and Chen, 2014; Price and Schwartz, 2008; Tan et al., 2010; Zhong and Jang, 2014). Among them, LC-based sensors constructed at the aqueous/LC interface have attracted particular attention for investigating enzymatic reactions (Bi et al., 2009; Brake et al., 2003, 2005; Hu and Jang, 2012a, 2012b; Park et al., 2006, 2008), whereas detection of the analytes is always only performed in the buffer system. This might limit utility of LC-based sensors for practical applications. Recently, we notice that an AChE LC biosensor for the detection of acetylcholine and the enzyme inhibitors was successfully demonstrated based on modulated growth of gold nanoparticles on the solid surface (Liao et al., 2012). However, this method could not be applied to monitor the activity of AChE and sensitivity of the sensor for detection of enzyme inhibitors is required to be improved for multiple applications. Therefore, it is still essential to

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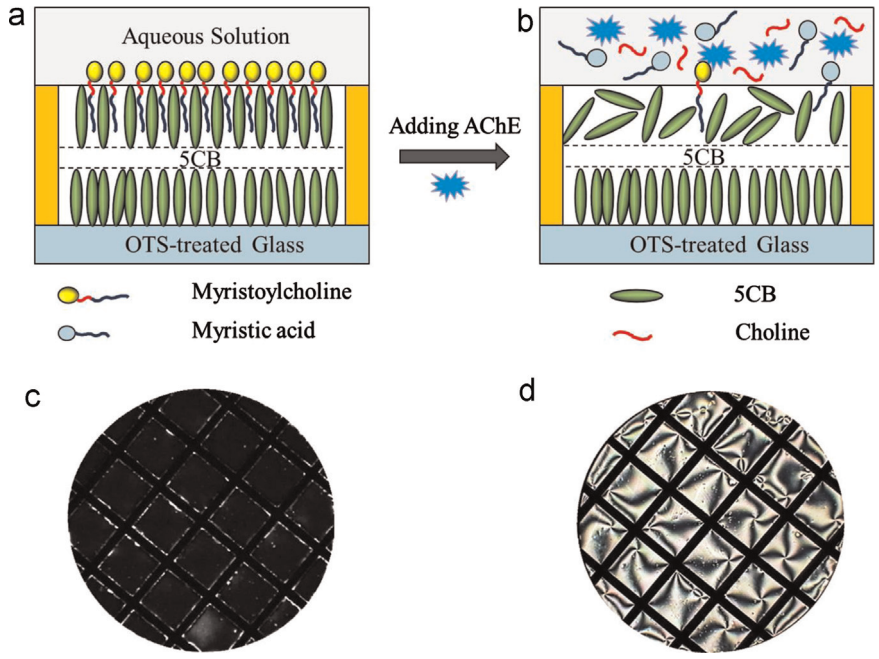

Fig. 1. Schmatic illustration of the orientational transition of LCs coupled to the enzymatic hydrolysis of Myr by AChE at the aqueous/LC interface: (a) perpendicular orientation and (b) planar orientation. The corresponding optical image associated with the orientation of LCs: (c) black appearance and (d) bright appearance.

develop a quite simple and convenient method that can monitor the activity of AChE and detect enzyme inhibitors with high sensitivity.

In this study, we developed a label-free strategy based on a cationic surfactant-decorated LC interface to monitor the enzymatic activity of AChE in the buffer and serum albumin solution, respectively. Enzymatic hydrolysis of the self-assembled monolayer of the cationic surfactant, myristoylcholine chloride (Myr), induced transition of LCs from perpendicular (Fig. 1a) to planar (Fig. 1b) orientation at the interface of aqueous solution and LCs, which changes the optical appearance of LCs from black (Fig. 1c) to bright (Fig. 1d). Effect of the inhibitor with different concentrations on the enzyme was also investigated in this study. Compared to exiting techniques, this simple, convenient and low-cost sensing device does not rely on complex instrumentation or intensive labor, which enables label-free screening of AChE and its inhibitors with high sensitivity.

2. Experimental

2.1. Materials

Acetylcholinesterase (AChE), phosphate buffered saline (PBS) (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl; pH=7.4) were purchased from Sigma-Aldrich. Myristoylcholine chloride (Myr) was obtained from Shanghai Shifeng Biological Technology Co., Ltd. Copper specimen grids (50 mesh, pitch=500 μm, bar=80 μm, hole=420 μm) were purchased from GILDER. Nematic liquid crystal 4-cyano-4′-pentylbiphenyl (5CB), octyltrichlorosilane (OTS), heptane, neostigmine bromide, were purchased from J&K Scientific Co., Ltd. Sulfuric acid, hydrogen peroxide (30% w/v), and bovine serum albumin (BSA) were obtained from Shandong Aibo Technology Trade Co., Ltd. Cellulase was purchased from Beijing Jingke Hongda Biotechnology Co., Ltd. The buffers with no salt and with additional salt were constituted by 10 mM phosphate (pH=7.4) and 10 mM PBS (pH=7.4) with additional 500 mM NaCl, respectively.

2.2. Treatment of glass microscope slides

The OTS-coated glass slides were prepared following the previous literatures (Brake and Abbott, 2002; Fletcher et al., 2009; Hu and Jang, 2012a, 2012b). Briefly, the glass microscope slides were first immersed in "piranha solution" (70% H_2SO_4/30% H_2O_2, caution: "piranha solution" reacts violently with organic substance 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 thoroughly with water, ethanol, and methanol, and then dried under a steam of gaseous N_2, followed by heated to 120 °C overnight. The "piranha-cleaned" glass slides were immersed in the OTS/heptane solution for 30 min. Then, they were rinsed with methylene chloride and dried under a stream of N2.

2.3. Preparation of optical cells

Copper specimen grids were first put onto the OTS-treated glass slides. Then, ~1 μL of 5CB that was 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 5CB on the grid. Subsequently, 200 μL aqueous solutions of interest were introduced into optical cell at room temperature. All the results were repeated at least three times.

2.4. Examination of optical images

A polarized light microscope (XPF-800C, Tianxing, Shanghai, China) was used to acquire the optical image of 5CB. All images were obtained by a 2.5 × objective lens and a digital camera (TK-9301EC, JVC, Japan) at room temperature. The direction of the polarized light is perpendicular to the sample stage. Bright area coverage ratios (B_r) of the optical images were obtained via calculating pixels of the bright area divided by pixels of the integral LC region using the Photoshop Software.