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Acetylcholinesterase Liquid Crystal Biosensor Based on Modulated Growth of Gold Nanoparticles for Amplified Detection of Acetylcholine and Inhibitor

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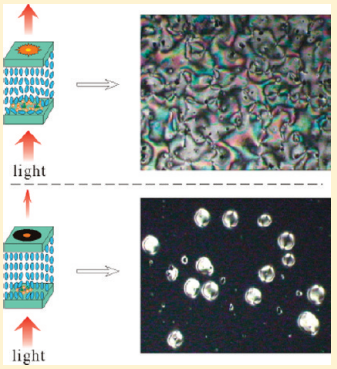
Supporting Information

ABSTRACT: A novel acetylcholinesterase (AChE) liquid crystal (LC) biosensor based on enzymatic growth of gold nanoparticles (Au NPs) has been developed for amplified detection of acetylcholine (ACh) and AChE inhibitor. In this method, AChE mediates the hydrolysis of acetylthiocholine (ATCl) to form thiocholine, and the latter further reduces $AuCl_4^-$ to Au NPs without Au nanoseeds. This process, termed biometallization, leads to a great enhancement in the optical signal of the LC biosensor due to the large size of Au NPs, which can greatly disrupt the orientational arrangement of LCs. On the other hand, the hydrolysis of ATCl is inhibited in the presence of ACh or organophosphate pesticides (OPs, a AChE inhibitor), which will decrease the catalytic growth of Au NPs and, as a result, reduce the orientational response of LCs. On the basis of such an inhibition mechanism, the AChE LC biosensor can be used as an effective way to realize the detection of ACh and AChE inhibitors. The results showed that the AChE LC biosensor was highly sensitive to ACh with a detection limit of 15 μmol/L and OPs with a detection limit of 0.3 nmol/L. This study provides a simple and sensitive AChE LC biosensing approach and offers effective signal enhanced strategies for the development of enzyme LC biosensors.



Liquid crystals (LCs) possess unique liquid-crystalline phase properties, which makes them ideal candidates for developing novel sensing systems with advanced and powerful functions. The orientation of LC molecules is sensitive to physical and chemical properties of a bounding interface, and the long-range order inherent and optical anisotropy can transform chemical and biomolecular binding events into amplified optical signals. Since the Abbott group[1] initiated the detection of biological events using LCs as sensing elements, LC based biosensors have attracted increasing interest in biological application. The LC based sensing detections can be carried out in ambient light without the need for electrical power or molecule labels, and their optical signals can be easily observed, even with naked eye,[1−5] making them well suited for the direct transduction, high sensitivity, and low-cost bioassays performed away from central laboratories.[6−9] Owing to these superior properties, many efforts have been taken to the development of LC biosensors for the specific biomolecular binding events, such as protein−ligand, protein−protein, and nucleic acid hybridization recognition events.[1,2,6−11] However, their optical signal sensitivity in these LC biosensing systems based on the biomolecular binding events generally depends on the size and amount of macro-biomolecules. Few LC biosensor examples have been reported for the detection of enzymatic events until now,[3,10,12,13] because most of the enzymatic events just catalyze substrate to generate some small-molecule products, whose disruption behavior to the orientational arrangement of LC molecules is far smaller than that of macro-biomolecules.

The enzymatic growth of metal nanoparticles is an interesting field and some creative applications as signal amplification elements in biosensors have been described. For example, alcohol dehydrogenase can modulate the deposition of copper onto gold seeds which amplifies the optical[14,15] and chronocoulometric[16] detection of alcohol by changing the shape or size of gold nanoparticles (Au NPs). Glucose-oxidase[17] is used to mediate catalytic deposition of gold on the Au NP seeds for sensitive optical detection of glucose. Another approach used hydrolase to stimulate the catalytic enlargement of Au NP seeds for the amplified colorimetric assays in the detection of the inhibitor[18] and tyrosinase activity.[19] Recently, our group[20] reported a signal-enhanced LC DNA biosensor by introducing an enzymatic metal deposition process. It is found that the deposition of silver nanoparticles is greatly benefited to change the surface topology of LC sensing interface and causes an enhancing disruption to the LC orientation, resulting in a significant decrease in the detection limit from 1 nmol/L to 0.1 pmol/L. In view of the optical amplification of enzymatic metal nanoparticles for the LC DNA biosensor, we believe that the exploitation of enzymatic growth of metal nanoparticles can

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also lead to the development of effective LC biosensors for enzymatic events.

The stimulated hydrolysis of acetylcholine (ACh, a neurotransmitter) into choline by acetylcholinesterase (AChE) plays an important role in brain chemistry.[21] The detection of ACh is of great clinical importance.[22] Inhibition of AChE by the enzyme inhibitors, such as organophosphate pesticide (OPs), can lead to the accumulation of ACh throughout the body, affecting the physiology of the nervous system with serious or fatal consequences.[23] In the past decade, many efforts have been taken to develop sensitive and efficient AChE biosensing systems for the detection of ACh and AChE inhibitors.[24−27] Even so, there is still significant interest in seeking new biosensing strategies to develop high flexibility of AChE biosensing systems.

Recently, we noticed that the AChE can be used to mediate the formation of Au NPs without adding gold nanoseeds.[28] The AChE molecules can catalyze the hydrolysis of ATCl to form thiocholine, which reduces $AuCl_4^-$ to a product of Au NPs. In this paper, we try to construct a new LC biosensing system based on the enzymatic growth of Au NPs for the detection of ACh and AChE inhibitor. To our best knowledge, this is the first example of the LC enzymatic biosensing system using enzymatic growth of nanoparticles to enhance the optical signal of LCs. In this system, the small-molecule products of AChE catalyzation, thiocholine molecules, are transferred to large-sized Au NPs, which can remarkably change the orientation of LCs, leading to an amplified optical signal and further improving the detection sensitivity of the LC enzymatic biosensor greatly.

The design and process of AChE LC biosensors based on enzymatic deposition of Au NPs is depicted in Figure 1b−d. First, a plain glass slide surface is chemically functionalized by self-assembling a thiethoxysilybutylaldehyde/*N*,*N*-dimethyl-*N*-octadecyl(3-aminopropyl) trimethoxysilyl chloride (TEA/DMOAP) film (Figure 1b). The AChE immobilization is then achieved by binding the amino groups of AChE with the aldehyde groups on the TEA/DMOAP film (Figure 1c). Finally, the glass slide modified with AChE is immersed in an Au NPs growth solution containing 1.2 mmol/L $HAuCl_4$ and 3 mmol/L ATCl. The AChE can mediate the hydrolysis of ATCl to form thiocholine. The latter, in turn, reduces $AuCl_4^-$ to Au^0, which further forms Au NPs on the glass slide (Figure 1d). Detailed experimental processes are listed in the Supporting Information.

Previous experimental studies have revealed that changes of either the chemical composition or topographical structure of the surface close to LCs may result in corresponding changes in the orientation of LCs.[29] In this LC biosensing system, the different surface modification of LC cells plays a key role in the orientation of LCs. The TEA/DMOAP mixed monolayer on the glass slide of LC cells can effectively induce homeotropic alignment of LC molecules (Figure 1b′), and as shown in Figure 2a, a uniform dark background optical image is observed through a polarized light microscope in transmission mode under a crossed polarizer. When the low concentration of AChE is immobilized on the TEA/DMOAP film, only a slight disruption to the homeotropic alignment of LC molecules is formed (Figure 1c′). Figure 2b shows that the optical image is not changed but a few of bright spots when the glass slide is modified with 10^{-4} U mL^{-1} of AChE. After immersing the AChE modified substrate into the enzymatic growth solution of Au NPs contained 3 mmol/L ATCl and 1.2 mmol/L $HAuCl_4$;

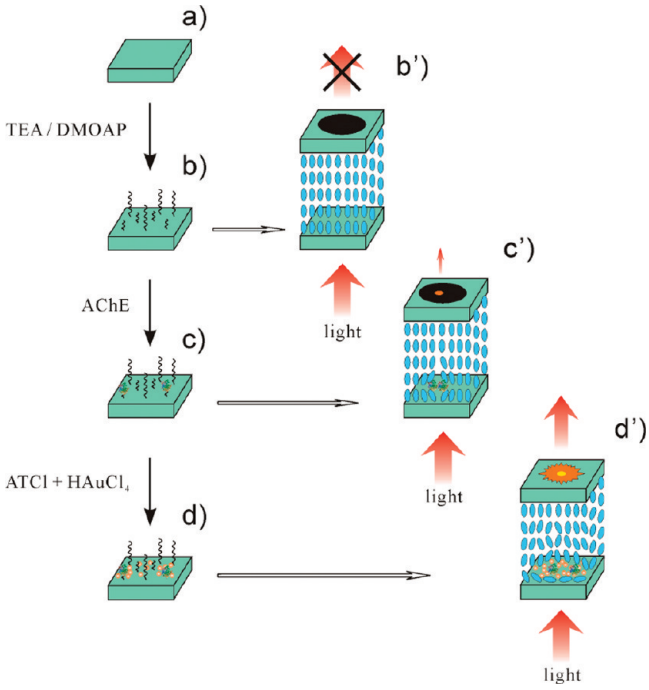

Figure 1. Stepwise assembly of the plain glass slides (left) and the orientation of 5CB in the cells (right) fabricated with DMOAP-coated glass slides (upper) and modified slides (lower): (a) cleaned glass slide, (b and b′) self-assembled TEA/DMOAP film, (c and c′) immobilization of AChE, and (d and d′) Au NPs obtained through biocatalytic growth.

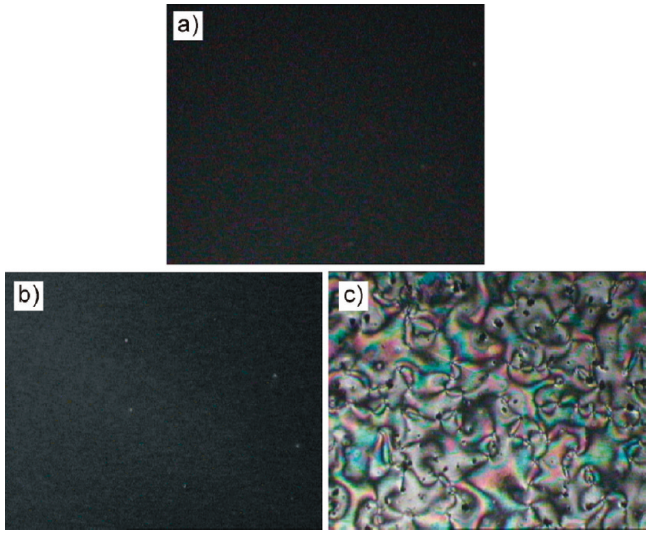

Figure 2. Optical images under cross polarizer of LC cells with 5CB in different conditions: (a) self-assembled TEA/DMOAP film, (b) immobilization of AChE (10^{-4} U mL^{-1}), (c) Au NPs obtained through biocatalytic growth.

however, the resulting large-sized Au NPs can greatly change the surface topology and induce a homeotropic-to-tiled transition of the LC molecules around (Figure 1d′). The corresponding optical image of the modified LC cell displays an obvious birefringent result as shown in Figure 2c. These results suggest that the Au NPs based on AChE enzymatic growth can remarkably enhance the optical signal of the LC biosensing system, and it is feasible to construct a highly sensitive LC biosensing system for enzymatic events.

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Thiocholine as a reductant for the catalytic growth of Au NPs without nanoseeds can be further supported by scanning-electron microscopy (SEM) (see the Supporting Information, Figure S1a,b). The SEM images also suggest that different surface topologies of the glass substrate can be achieved before and after AChE enzymatic deposition of Au NPs.

Since the enzymatic deposition of Au NPs plays an important role in the optical signal amplification of LC enzymatic biosensing system, it is necessary to choose a suitable AChE concentration to ensure that the enzymatic deposition of Au NPs is sufficient to cause remarkable optical signal while the bound AChE hardly changes the surface topology. The experimental results show that the number of bright spots in the optical images decreases with the AChE concentration from 10^{-2} U mL^{-1} down to 10^{-4} U mL^{-1}, where almost a uniform dark background appears (see the Supporting Information, Figure S2). Thus, 10^{-4}U mL^{-1} AChE is used in the subsequent section. Incubation time for enzymatic deposition of Au NPs is also an important factor. Prior to the optical images, the AChE modified substrates were incubated in the growth solution of Au NPs containing 3 mmol/L ATCl and 1.2 mmol/L $HAuCl_4$ from 0.5 h to ~2.0 h. The SEM results (see the Supporting Information, Figure S3) show that both the size and the amount of Au NPs increase with the incubation time, which benefits the change of the surface topology of the LC cell and induces a homeotropic-to-tiled transition of the LC molecules around. It can be seen that the birefringent textures in the optical appearance of LCs increase with incubation time for enzymatic deposition of Au NPs (see the Supporting Information, Figure S4). When the response time further increases, the birefringent textures in the optical appearance of LCs have no discernible difference. To obtain the best optical appearance of LCs and the least incubation time, a 1.5 h incubation time is used in the latter experiment.

The AChE as a hydrolase can stimulate the hydrolysis of ACh and ATCl. We have described that the hydrolysis of ATCl results in a reductant, thiocholine, which can further reduce $AuCl_4^-$ to form Au NPs, an excellent signal enhancement element in the AChE LC biosensing system. When ACh and ATCl competitively combine with AChE, the catalytical hydrolysis of ATCl to obtain thiocholine is inhibited and the subsequent reduction of $AuCl_4^-$ to produce Au NPs is also decreased. As a result, the disruption to the orientation of LC molecules is reduced, which decreases the optical signal of LC biosensor (see the difference in inserts in parts a and c of Figure 3). On the basis of this competitive principle, a novel signal amplified LC biosensor is constructed for acetylcholine.

In this work, the detection of ACh is achieved by establishing the relationship between the ACh concentrations and the optical images of birefringent texture. A set of AChE-decorated glasses are immersed in an Au NPs growth solution (consisting of 1.2 mmol/L $HAuCl_4$ and 1.5 mmol/L ATCl) at 37 °C for 1.5 h, in which 0, 0.015, 0.15, or 1.5 mmol/L ACh is added to, respectively. If no ACh is added, the optical image displays obvious birefringent texture. However, the optical images of birefringent texture decrease by degrees with the increase in the ACh concentration from 0.015 to 1.5 mmol/L. When the ACh concentration reaches 1.5 mmol/L, only some weak bright spots can be observed, indicating that the ACh greatly inhibits the hydrolysis of ATCl to obtain thiocholine and few Au NPs with large dimension size are formed. These gradually changed results suggest that the LC based AChE biosensing system can contribute a simple method for the detection of ACh (the detection limit is ~15 μmol/L). The precision of the LC biosenor was performed by a three-repetitive measurement of the concentration of ACh from 15 μmol/L to 1.5 mmol/L. The resulted birefringent textures for the same concentration are similar. This outcome reflects the good reproducibility of the LC biosensor for ACh. Moreover, when the as-prepared liquid crystal cells was not in use, they were stored at room temperature. No obvious differences in birefringent textures were observed after several days' storage, indicating the acceptable stability of the biosensor.

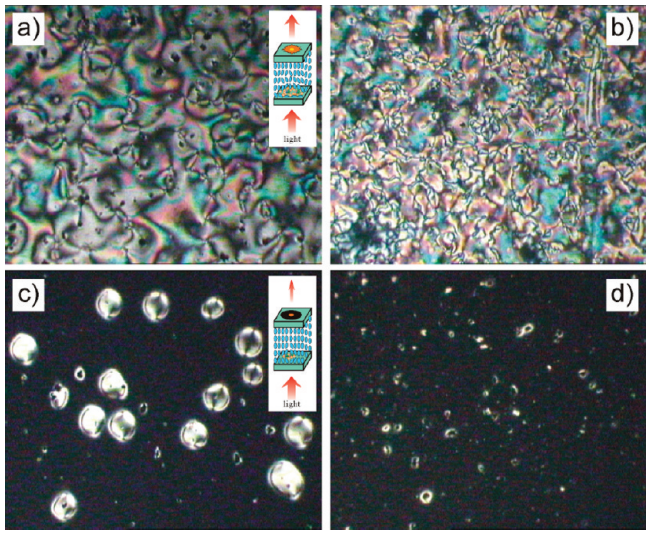

Figure 3. Analysis of acetylcholine (ACh) by ACh and ATCl competitive combination with AChE based on the liquid crystal biosensor. Optical images under the cross polarizer of LC cells with 5CB were obtained at different concentrations of acetylcholine (ACh): (a) 0, (b) 0.015, (c) 0.15, and (d) 1.5 mmol/L. The insert in parts a and c show the scheme for the orientation of 5CB in the cell after biacatalytic growth of Au NPs without and with competitive ACh.

Organophosphate pesticide (OP) is known as an inhibitor to inhibit the reaction between AChE and the substrate. It exerts inhibitory effect through irreversibly attacking the serine of peptide located at the active site of AChE to form a stable phosphorylated adduct (OP-AChE).[30] When organophosphate exists, the thiocholine product resulting from the AChE catalyzation hydrolysis is reduced, which decreases the amount of Au NPs deposited on the glass slide. Therefore, the disruption to the orientation of LC molecules is reduced (see the difference of the inserts in parts a and e of Figure 4), making less birefringent texture domains in the optical images of the LC cell. On the basis of this irreversible inhibition principle, a novel signal amplified LC biosensor is constructed for analyzing organophosphate pesticide.

The detection of different concentrations of OPs is obtained by the birefringent degree of the optical images. The AChE decorated glasses are first immersed in a PBS buffer solution containing different concentrations of standard malathion at 38 °C for 25 min, washed with a solution of propanone and water (1:19 v/v), dried under nitrogen atmosphere, then immersed in Au NPs growth solution consisting of 1.2 mmol/L $HAuCl_4$ and 1.5 mmol/L ATCl at 37 °C for 1.5 h. Experiment results reveal that if there is no OPs inhibition, the optical image displays obvious birefringent texture (Figure 4a). However, the birefringent texture domains in the optical images of LC cell decrease with the concentration of OPs from 0.3 to 3000 nmol/L as shown in Figure 4b–f. When the OP concentration

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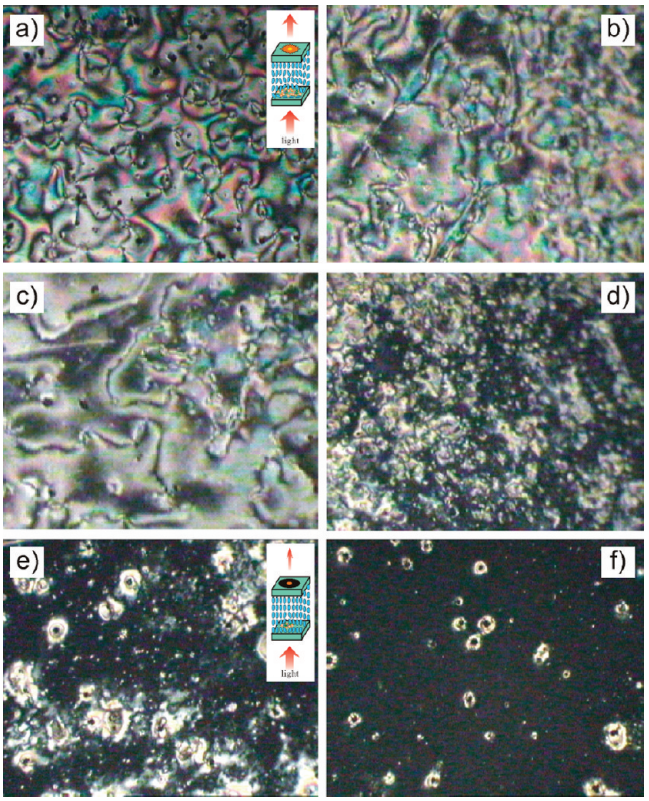

Figure 4. Analysis of organophosphate pesticide (OPs) by inhibiting the reaction between AChE and ATCl based on the liquid crystal biosensor. Optical images under the cross polarizer of LC cells with 5CB were obtained at different concentrations of organophosphate pesticide (OPs): (a) 0, (b) 0.3, (c) 3, (d) 30, (e) 300, and (f) 3000 nmol/L. The inset in part a shows the scheme for the orientation of 5CB in the cell without the inhibition of OPs, and the inset in part e shows the scheme for the orientation of 5CB with the inhibition of OPs.

reaches 3000 nmol/L, only some weak bright spots can be observed. Therefore, it is clear that the simple LC based enzymatic biosensing method can provide a sensitive detection for organophosphate pesticide inhibitor (the detection limit is ~0.3 nmol/L). Such a low limit indicates that the present amplified LC sensor possesses a comparable sensitivity for OP detection as compared to the amplified electrochemical OP sensors recently developed.[28,31,32] A series of three-repetitive measurements of the concentration of OPs from 0.3 to 3000 nmol/L was used to estimate the precision. The result shows similar birefringent textures for the same concentration.

In summary, a novel AChE LC biosensor has been developed based on modulating the growth of Au NPs for amplified detection of ACh and AChE inhibitor (OPs). The enzymatic deposition of Au NPs changed the surface topology greatly, resulting in an obvious change of the optical appearances of LC biosensors from a dark background to a birefringent texture before and after enzymatic Au NPs. The proposed LC biosensor reveals that the enzymatic deposited gold nanoparticles are excellent signal enhancement elements in the detection of ACh and AChE inhibitor (OPs). With this innovative assay, it is able to detect acetylcholine with a concentration as low as 15 μmol/L and organophosphate pesticide with a detection limit of 0.3 nmol/L. The new approach possesses the attractive performance such as the visualization of the output, simplicity of the procedure, and a comparable sensitivity for OPs detection as compared with the electrochemical OPs sensors recently developed. To our knowledge, this is the first demonstration of the LC biosensor for the detection of ACh and the AChE inhibitor. This study also offers an effective signal amplification strategy to develop highly sensitive LC enzymatic biosensors.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedure and supplementary SEM and optical images. This material is available free of charge via the Internet at http://pubs.acs.org.

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