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Gold nanoparticle based signal enhancement liquid crystal biosensors for tyrosine assays

Xian Li, Guang Li, Meng Yang, Long-Cong Chen, Xing-Liang Xiong*

Laboratory of Bioengneering, Chongqing Medical University, Chongqing 400016, People's Republic of China

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ABSTRACT

A novel liquid crystal (LC) biosensor based on seedless production of gold nanoparticles (AuNPs) has been developed for the detection of L-tyrosine (Tyr). In this study, tyrosinase (TR) stimulated the biocatalyzed hydroxylation of Tyr to L-DOPA, which mediated the generation and growth of AuNPs. The large-size of AuNPs greatly changed the surface topology of LC sensing interface, the corresponding images were regulated under MATLAB, which allows the quantitative detection of Tyr. Under the optimum conditions, the limit of Tyr detection could be as low as 2×10^{-7} mol/L. Results also showed that phenylalanine (Phe), tryptophan (Trp) and threonine (Thr) caused no interference. This method provides a simple, label-free and sensitive strategy for determination of Tyr.

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1. Introduction

Liquid crystals (LC), intermediate between a state of crystalline and an isotropic liquid [1], possess many of the mechanical properties of liquid, such as high fluidity and coalescence of droplets [2]. In addition, they are similar to crystals in that they exhibit anisotropy in their optical, mechanical, electrical, and magnetic properties [3]. LCs present a long-range orientational order in the arrangement of constituent molecules, and exhibit a great variety of phases, which are very sensitive to minute changes on the surface [4]. These orientational responses can be amplified to tens of micrometers away [5]. Moreover, the orientational changes of LCs can be readily observed under crossed polarizers account for its unique birefringent, and the whole procedure can be carried out under ambient light evenly without electrical power [6]. The LC-based system is a promising platform for chemical and biological sensing which can be used for real-time and label-free detection with high sensitivity and without any complex instrumentation [7]. In 1998, Abbott and coworkers [8] initiated the field of using LCs as sensing elements to transducer and amplify molecular events, which was a landmark in the detection of biomolecules. Several past studies have demonstrated that LCs has been used for investigating enzymatic activities [9,10], organophosphonates detection [11–13], and protein–protein binding events [14].

L-Tyrosine (Tyr), a necessary amino acid for human, plays an important role in signaling pathways of cellular functions, and dopamine and adrenaline are derived from this amino acid [15]. Furthermore, the activity of Tyr directly regulates the melanin's absorption, which can be used to treat albinism. So the detection of Tyr is of great clinical importance [16]. However, in comparison with the existing methods including AccQ-Tag [17], potentiometric titration [18] and HPLC [19] still have some drawbacks such as the poor specification using AccQ-Tag method, the unsatisfied limit of detection (LOD) and ambiguous end-point of potentiometric titration, high-cost and long-term HPLC. Thus, it is necessary to seek new biosensing strategies to develop a flexible system that could overcome the above flaws.

Willner and co-workers [20] have reported that neurotransmitters mediate the generation and growth of AuNPs, and they also use this process for the quantitative assay of enzyme activity. This work has attracted a particular attention, because the formation of AuNPs in the systems proceeds without AuNP seeds, which illuminate an entirely new thought using enzymatic growth of AuNPs. Similarly, we can adapt this process as the signal amplified mechanism for LC biosensor. As the orientation of LCs is extremely sensitive to large molecules, these aggregated nanoparticles can greatly change the surface topology, and result in significant changes in correspondence of optical images, which is examined by a polarized light microscopy [21]. In this study, we aim to explore a sensitive and signal-enhanced LC biosensor based on enzymatic AuNPs deposition, and the system has been developed to investigate the AuNPs deposition in response to the orientation of LCs, results were

* Corresponding author. Tel.: +86 18084014053.
E-mail address: xlxiong@cqmu.edu.cn (X.-L. Xiong).

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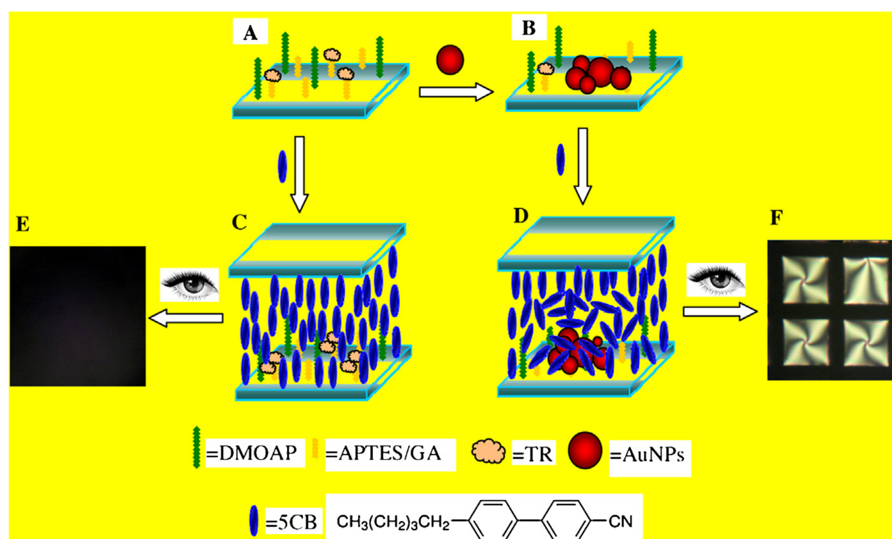

Scheme 1. Illustration diagram of detection strategy of LC biosensor: (A) cimmobilization of TR, (B) deposition of AuNPs through the enzymatic process, (C) homeotropic orientation of 5CB in the cells without AuNPs on glass slides, (D) the disrupted orientation of 5CB in the cells with AuNPs deposited on glass slides. Photograph under polarized light microscope (E, F).

regulated under MATLAB, which allows the quantitative detection of Tyr.

2. Materials and methods

2.1. Reagents and materials

L-Tyrosine (Tyr), tyrosinase (TR), (3-aminopropyl) triethoxysilane (APTES), N,N-dimethyl-N-octadecyl (3-aminopropyl) trimethoxysilyl chloride (DMOAP), Phosphate buffer solution (PBS) were purchased from Sigma–Aldrich (St. Louis, USA). LC 4-cyano-4′-pentylbiphenyl (5CB) was obtained from Huajing Scientific and Technological Development Co., Ltd. (Hebei, China). $HAuCl_4{\cdot}4H_2O$ was purchased from Jiuling Chemical Co., Ltd. (Shanghai, China). Glutaraldehyde (GA) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). $CuCl_2{\cdot}2H_2O$ was obtained form Tianmao Chemical Co., Ltd. (Tianjin, China). Glass slides were provided by Xinhua Laboratory Glassware Company (Haimen, China). All the reagents were used as received without further purification.

2.2. Design of biosensor

As shown in Scheme 1, the surface was fabricated using (APTES + GA)/DMOAP mixture, which not only provided surface amino groups for coupling TR, but also orientated LCs homeotropically to yield a dark background. Afterwards, AuNPs growth solution was added to the TR immobilized surface, TR stimulated the biocatalyzed hydroxylation of Tyr to L-DOPA which could reduce Au^{3+} in growth solution into AuNPs in the system. As lack of a stabilizer it was possible to monitor the growth of AuNPs into small nanoclusters and as time went by aggregates of nanoparticles were formed. The amount of these particles of various shapes and sizes settled down to the glass slides that could greatly change the surface topology and induce a homeotropic-to-tiled transition of the LC molecules, resulting in a significant change in corresponding of optical appearances under the crossed polarized light.

2.3. Cleaning of substrates

The glass slides were firstly cut into size of 2 cm × 2 cm square, and then cleaned with freshly prepared piranha solution (70% H_2SO_4, 30% H_2O_2) at 80 °C for 2 h to remove all organic contaminants, followed by rinsing with copious amount of deionized water, drying under a stream of nitrogen (N_2) and heating at 110 °C at least 3 h.

2.4. APTES/DMOAP-decoration

Clean glass slides were immersed in ethanol solution containing 5% (v/v) APTES and 1% (v/v) DMOAP at 80 °C for 2 h. After washing the slides three times with ethanol and deionized water, the slides were dried under N_2, heated at 110 °C for 1 h. Subsequently, the slides were immersed in a 0.3 mol/L GA solution [22,23] for 30 min and rinsed with deionized water, dried with nitrogen flow.

2.5. Tyrosinase immobilization

Tyrosinase was firstly dissolved in PBS buffer (0.1 mol/L, pH 7.4) and prepared to 3×10^{-4} U/mL. And then dip the solution on glass slides for 1 h at ambient temperature (25 ± 2 °C). The slides were cleaned sequentially in PBS buffer (0.1 mol/L, pH 7.4), deionized water, and dried with nitrogen flow.

2.6. Nanoparticle growth solution

Growth solutions consisted of $HAuCl_4$ (2×10^{-4} mol/L), in PBS buffer (0.1 mol/L, pH 7.2), and different concentrations of Tyr and $CuCl_2$. All solutions were used within 2 h after preparation and the experiment were performed at room temperature (28 °C). The samples were prepared by placing a drop of nanoparticle growth solution on a TR-coated glass slide, washed with PBS buffer (0.1 mol/L, pH 7.4), deionized water and subsequent nitrogen-drying.

2.7. Preparation of optical cells

The LC cells were fabricated by spacing a clean copper grid between a prepared glass and a clean glass slide. A copper grid was firstly placed on a decorated glass, then dipping 5CB to fill the grid. After that, a clean glass was carefully covered on it to form an LC layer of 20 μm in thickness. Lastly, install several retainer clips.

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