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Enhanced performance of a hyperbranched rolling circle amplification based electrochemiluminescence aptasensor for ochratoxin A using an electrically heated indium tin oxide electrode

Huifang Zhang[a,b], Zesheng Zhuo[a], Lijuan Chen[a], Chaoqun Chen[a], Fang Luo[a,c,*], Yiting Chen[d,**], Longhua Guo[a], Bin Qiu[a], Zhenyu Lin[a,**], Guonan Chen[a]

[a] MOE Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Institute of Nanomedicine and Nanobiosensing, College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116,China

[b] School of Chemistry and Chemical Engineering, Key Laboratory of Organo-pharmaceutical Chemistry of Jiangxi Province, Gannan Normal University, Ganzhou 341000, China

[c] College of Biological Science and Engineering, Fuzhou University, Fuzhou, Fujian 350116, China

[d] Chemistry and Chemical Engineering Department, Minjiang University, Fuzhou, Fujian 350108, China

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ABSTRACT

A promising and novel electrochemiluminescence (ECL) aptasensor equipped with an electrically heated indium tin oxide (ITO) electrode was constructed. Temperature control was achieved by heating the ITO electrode, avoiding the tedious operation and substantial increase in noise experienced when heating the bulk solution. Using the heated ITO electrode, an order of magnitude improvement in ECL sensitivity was obtained by operating the electrode at 65 °C compared to 25 °C, and this can be used to further enhance the performance of the biosensor.

1. Introduction

Electrochemiluminescence (ECL) is a chemiluminescent reaction triggered by electrochemical processes. Instruments for detecting ECL are characterized by their simplicity and wide detection range [1], and this technique has been used together with hyperbranched rolling circle amplification (HRCA) and highly specific aptamers to develop many sensitive ECL aptasensors for a range of targets [2,3]. In these biosensors, the temperature needs to be controlled carefully during the DNA hybridization and enzyme reactions involved in HRCA procedures. Since different temperatures are required at each step, additional temperature control devices are needed for bulk solution heating and the rate of temperature change is slow. It is necessary to find some way to address this problem.

Electrode heating has been utilized for temperature control in the field of thermoelectrochemistry through microwaves [4], light beams [5], microheaters [6] and electrical heating [7]. Among these, electrically heated electrodes have attracted particular interest because the temperature of the electrode can be controlled easily through electrical heating while leaving the bulk solution unchanged [8]. In earlier research, great efforts have been made to employ heated electrodes to control the temperature for DNA interaction and enzyme activity [9–11]; satisfactory results were obtained in these studies. Moreover, this technique has been successfully used in the development of many sensitive electrochemical biosensors as the temperature greatly affects the reaction rate and diffusion rate of electroactive compounds on the electrode surface [12]. The ECL performance of luminol [13] and Ru $(bpy)_3^{2+}$ [14], for example, are greatly affected by the electrode temperature. Furthermore, the effect of temperature on enzyme reactions has been studied using a heated electrode [15]. However, in the experiments mentioned above the ECL indicator operated in a dynamic way. To further investigate the effects of electrode temperature on ECL performance, an immobilized ECL indicator was introduced.

In this study, an electrically heated electrode was utilized as the temperature controller in a novel ECL aptasensor. Ochratoxin A (OTA), a ubiquitous mycotoxin generated by fungi in the process of food storage that can damage the function of the kidneys, liver and immune system [16,17], was chosen as the model target. The results showed that the ECL performance of the biosensor was improved by heating the electrode to the optimal temperature during the ECL detection process. Moreover, electrical heating has a number of advantages compared to bulk solution heating, including a faster rate of heating, simpler

* Correspondence to: F. Luo, College of Biological Science and Engineering, Fuzhou University, Fuzhou, Fujian 350116, China.
** Corresponding authors.
E-mail addresses: luofang@fzu.edu.cn (F. Luo), cyt@mju.edu.cn (Y. Chen), zylin@fzu.edu.cn (Z. Lin).

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operation and more sensitive detection.

2. Experimental section

2.1. Instruments and reagents

A laboratory-built ECL detection system integrated with a heated ITO electrode (described more fully elsewhere [14]) was used in this study.

The T-shaped ITO conductive glass (2 cm × 2 cm slice with a T-shape ITO membrane 4 mm wide) was purchased from Xiangcheng Technology Co. (Shenzhen, China). *Escherichia coli* (*E. coli*) DNA ligase, Bst DNA polymerase, bovine serum albumin (BSA), magnesium sulfate ($MgSO_4$) and deoxynucleotide solution mixture (dNTPs) were acquired from Takara Biotechnology Co., Ltd. (Dalian, China). Chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$), dichlorotris (1,10-phenanthroline) ruthenium(II) hydrate ($Ru(phen)_3^{2+}$), Tripropylamine (TPA) and OTA were purchased from Sigma-Aldrich (Shanghai, China).

All of the designed oligonucleotides were synthesized by Sangon Inc. (Shanghai, China). Their sequences are given below.

OTA aptamer: 5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3′.
Capture probe DNA (CDNA): 5′-SH-TGT CCG ATG CTC CCT TTA CGC CAC CCA CAC CCG ATC-3′.
Padlock probe: 5′-TAA AGG GAG CAT CGG ACA TCT TGT ATC CTT TGG TTG AAA CTT CTT CCT TTC TTT CTT CGA TCG GGT GTG GGT GGC G-3′.
Primer 1: 5′-TTC AAC CAA AGG ATA CAA GA-3′.
Primer 2: 5′-ACT TCT TCC TTT CTT TCT TC-3′.

2.2. Fabrication of heated ITO electrode and temperature calibration

The heated ITO electrode was fabricated using previously published procedures [18]. Cyclic voltammetry (CV) (from −0.4 to +0.6 V) was then performed in $HAuCl_4$ (0.5 mM) to form a layer of gold on the working area. The temperature of the electrically heated ITO electrode was calibrated three times using the equilibrium electrode potential of a standard redox couple shifted with temperature change, as reported previously [19,20].

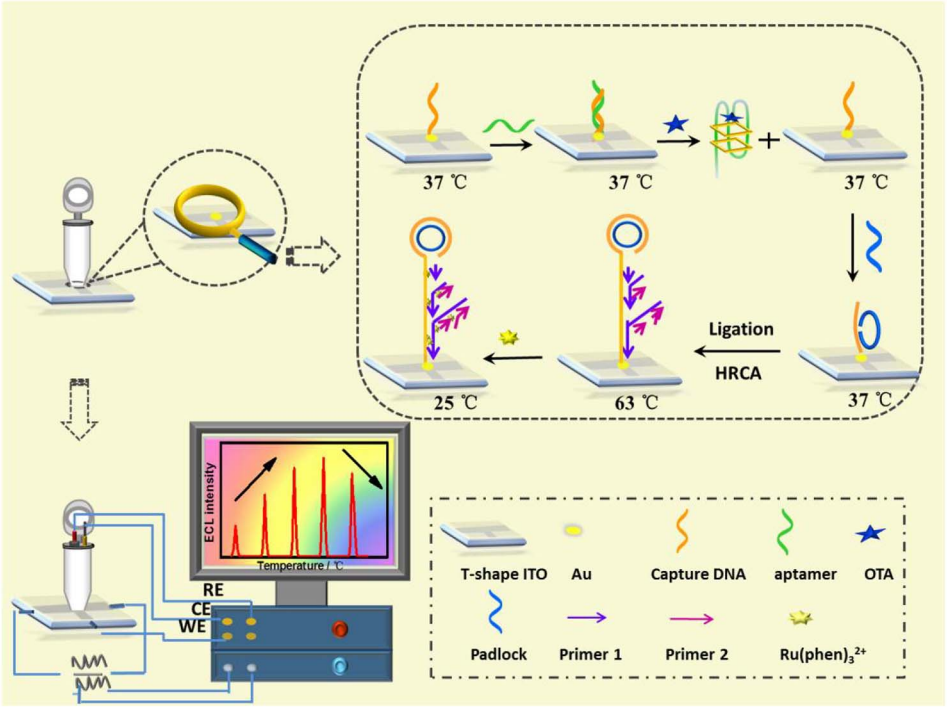
2.3. Fabrication of ECL aptasensor

Thiolated CDNA (1 μM) was added to the ITO electrode and incubated for 12 h in the dark. After blocking by MCH (1 mM), Tris-HCl buffer (50 mM, pH = 7.4) containing the aptamer (1 μM) was added to the above solution for incubation (37 °C, 1.5 h). After that, different concentrations of OTA were injected into the apparatus under the same conditions. The ligation reaction was carried out at an electrode surface temperature of 37 °C for 1 h in a solution consisting of padlock (1 μM) and *E. coli* DNA ligase (8 U). Next, the electrode was soaked in a solution containing original Bst DNA polymerase (2 μL), primer 1 (1 μM), primer 2 (1 μM) and dNTPs (0.9 mM) at 63 °C for 120 min. Finally, Ru $(phen)_3^{2+}$ (1 mM) was introduced into the reaction tube at room temperature for 3 h. After rinsing several times to remove the extra Ru $(phen)_3^{2+}$, PBS (10 mM, pH 7.4) containing 20 mM TPA was added to the reaction tube. The working electrode was electrically heated to the appropriate temperature and cyclic voltammetry (from 0.6 to 1.4 V, scan rate 0.1 V/s) was carried out to obtain the ECL signal.

3. Results and discussion

3.1. Principle of the proposed ECL biosensor

Scheme 1 shows the principle of the proposed biosensor. CDNA was initially immobilized on the electrode via a Au—S covalent bond. When the temperature of the electrode was adjusted to 37 °C, the aptamer hybridized with CDNA to form double stranded DNA (dsDNA). However, in the presence of the target, the aptamer preferred to form an OTA–aptamer complex instead of an aptamer–CDNA duplex, resulting in the release of aptamer from the ITO electrode surface [21]. The free CDNA could then hybridize with the terminal regions of the padlock at 37 °C to form a circular padlock. The HRCA reaction then occurred on the electrode surface, with a very large quantity of dsDNA fragments of variable length accumulating on the surface of the ITO electrode [2]. Ru $(phen)_3^{2+}$, used here as the ECL indicator, can intercalate into the



Scheme 1. Schematic representation of the proposed ECL aptasensor for sensitive detection of OTA using an electrically heated ITO electrode.