Analytica Chimica Acta 811 (2014) 70–75

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Ultrasensitive electrochemiluminescent aptasensor for ochratoxin A detection with the loop-mediated isothermal amplification

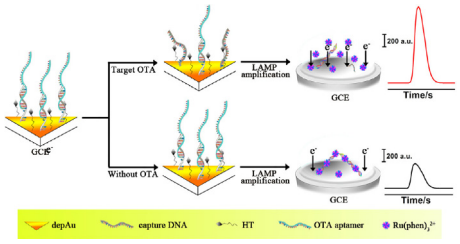
Yali Yuan[a,b], Shiqiang Wei[b], Guangpeng Liu[b], Shunbi Xie[a], Yaqin Chai[a,*], Ruo Yuan[a,*]

[a] Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China
[b] College of Resources and Environments, Southwest University, Chongqing 400715, PR China

HIGHLIGHTS

- We presented an ultrasensitive detection system for small molecule ochratoxin A.
- The loop-mediated isothermal amplification (LAMP) was employed here.
- The LAMP amplicons were readout by an electrochemiluminescence detection system.
- The quantitative analysis of OTA depended on the increase of ECL intensity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 September 2013
Received in revised form 2 November 2013
Accepted 8 November 2013
Available online 25 November 2013

Keywords:
Electrochemiluminescent
Loop-mediated isothermal amplification
Signal-on
Small molecule
Aptasensor

ABSTRACT

In this study, we for the first time presented an efficient, accurate, rapid, simple and ultrasensitive detection system for small molecule ochratoxin A (OTA) by using the integration of loop-mediated isothermal amplification (LAMP) technique and subsequently direct readout of LAMP amplicons with a signal-on electrochemiluminescent (ECL) system. Firstly, the dsDNA composed by OTA aptamer and its capture DNA were immobilized on the electrode. After the target recognition, the OTA aptamer bond with target OTA and subsequently left off the electrode, which effectively decreased the immobilization amount of OTA aptamer on electrode. Then, the remaining OTA aptamers on the electrode served as inner primer to initiate the LAMP reaction. Interestingly, the LAMP amplification was detected by monitoring the intercalation of DNA-binding $Ru(phen)_3^{2+}$ ECL indictors into newly formed amplicons with a set of integrated electrodes. The ECL indictor $Ru(phen)_3^{2+}$ binding to amplicons caused the reduction of the ECL intensity due to the slow diffusion of $Ru(phen)_3^{2+}$–amplicons complex to the electrode surface. Therefore, the presence of more OTA was expected to lead to the release of more OTA aptamer, which meant less OTA aptamer remained on electrode for producing LAMP amplicons, resulting in less $Ru(phen)_3^{2+}$ interlaced into the formed amplicons within a fixed $Ru(phen)_3^{2+}$ amount with an obviously increased ECL signal input. As a result, a detection limit as low as 10 fM for OTA was achieved. The aptasensor also has good reproducibility and stability.

1. Introduction

The amplified detection of targets with high sensitivity and accuracy is being greatly motivated by its potential applications in clinical diagnosis, food analysis, bioterrorism and environmental monitoring [1–4]. Recently appeared the loop mediated isothermal amplification (LAMP), which is an outstanding nucleic acid-based

* Corresponding authors. Tel.: +86 23 68252277; fax: +86 23 68252277.
E-mail addresses: yqchai@swu.edu.cn (Y. Chai), yuanruo@swu.edu.cn (R. Yuan).

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http://dx.doi.org/10.1016/j.aca.2013.11.022

exponential amplification procedure developed by Notomi [5]. The LAMP can simply amplify DNA with high efficiency, specificity and rapidity under isothermal conditions using a set of four specially designed primers (two inner and two outer primers) to recognize six distinct regions and a DNA polymerase with strand displacement activity incubated in a single tube [6]. Through alternating extension and strand displacement reactions, the entire LAMP process can continuously yield long DNA concatamers with prominent sensitivity, as upward of $\sim 10^9$ copies accumulate from less than 10 copies of input template within a hour [7–10]. Compared with traditional polymerase chain reaction, the LAMP offers superior advantages in terms of sensitivity, reaction speed, and amplicon yield, and can be applied to non-denatured genomic DNA samples under isothermal reaction conditions [11].

Until now, substantial specific DNA sequences have been successfully served as DNA templates and exponentially amplified by LAMP technique [12]. However, the base numbers of the specific DNA were all achieved in hundreds, which were limited to the detection of short DNA sequences. In order to overcome this shortcoming, Li et al. [13] adopted the target microRNA with few bases to serve as inner primer for initiating the strand displacement DNA synthesis based on the catalytic activity of RNA extension along a DNA template and the strand displacement activity of Bst DNA polymerase, which extended the examinable object in a certain extent, whereas this assay was just confined to the RNA detection. Up to now, the detection of protein, small molecule or metal ions based on the LAMP amplification has not been appeared to the best of our knowledge. The main problem is the lack of effectively signal translation between the target and LAMP amplification owing to the nucleic acid just suitable for LAMP process. Additionally, for the reported LAMP-based systems, the quantitative detection of LAMP products is mainly obtained by dye (ethidium bromide) staining, or by crudely monitoring the increase of either calcein fluorescence or solution turbidity due to the excessive release of pyrophosphate from nucleoside [14]. Despite real-time detection for these methods, the signals are due solely to the accumulation of base-pairs and can easily read false amplicons (molecular parasites) as true ones [15]. The electrochemical detection with redox probes interacted into formed amplicons, in part, solved the above issue. Nevertheless, the reported electrochemical systems were almost the signal-off platform and the sensitivity was limited [16–19].

Electrochemiluminescence (ECL) is a kind of chemiluminescence (CL) produced by an electrochemical reaction, which has been proven as a highly sensitive and selective detection method alternative to conventional electrochemical and CL techniques [20,21]. In this communication, by using the integration of LAMP techniques and subsequently direct readout of LAMP amplicons with an ECL detection system, we for the first time presented an efficient, accurate, rapid and ultrasensitive signal-on ECL assay to achieve the small molecule detection. Ochratoxin A (OTA), a potent toxin produced by several species of *Aspergillus ochraceus* and *Penicillium verrucosum* that grow in grains, coffee, cereals, dried fruits, beers and wines, was chose as target model [22]. In our work, a template DNA successively contained the sequences of B3, B2, B1, F1c, F2c and M was synthesized. And the sequence of M was perfectly complementary to the OTA aptamer (see Table 1). Firstly, the dsDNA composed by OTA aptamer and its capture DNA were immobilized on the electrode. After the target recognition, the OTA aptamer bond with target OTA and subsequently left off the electrode, which effectively decreased the immobilization amount of OTA aptamer on electrode. Then, the remaining OTA aptamers on the electrode served as inner primer to hybridize with template DNA and thus initiated the LAMP reaction based on the catalytic activity of OTA aptamer extension along the DNA template and the strand displacement activity of Bst DNA polymerase. Interestingly, the LAMP amplification was detected by monitoring the intercalation of DNA-binding $Ru(phen)_3^{2+}$ ECL indictors into newly formed amplicons with a set of integrated electrodes. The ECL indictor $Ru(phen)_3^{2+}$ binding to amplicons caused the reduction of the ECL intensity due to the slow diffusion of $Ru(phen)_3^{2+}$–amplicons complex to the electrode surface. Therefore, the presence of more OTA was expected to lead to the release of more OTA aptamer, which meant less OTA aptamer remained on electrode for producing LAMP amplicons, resulting in less $Ru(phen)_3^{2+}$ interlaced into the formed amplicons within a fixed $Ru(phen)_3^{2+}$ amount with an obviously increased ECL signal input (see Scheme 1).

2. Experimental

2.1. Chemicals and material

Dichlorotris(1,10-phenanthroline)ruthenium hydrate ($Ru(phen)_3Cl_2{\cdot}H_2O$), ochratoxin A (OTA), hexanethiol (96%, HT) and chloroauric acid ($HAuCl_4$) were obtained from Sigma–Aldrich (St. Louis, MO). Bst polymerase large fragments, betaine, $MgSO_4$ and the deoxynucleotide triphosphates (dNTPs) were purchased from New England Biolabs Ltd. (Beijing, China). Except for the template DNA from Integrated DNA Technologies, Inc. (IDT, USA), all the other oligonucleotides used in the present study were synthesized by Shanghai Shenggong Biotechnology Co. (Shanghai, China) and used without further purification (see Table 1).

Aptamer stock solutions were obtained by dissolving oligonucleotides in 20 mM Tris–HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$. 0.10 M sodium phosphate buffer (PBS, pH 7.0) containing 10 mM KCl, 2 mM $MgCl_2$ was used as working buffer solution. All other chemicals used in this investigation were of reagent grade unless mentioned otherwise. Ultrapure water was used throughout this study.

2.2. Fabrication of the ECL aptasensor

The GCE was first polished sequentially with 1.0 and 0.3 μm alumina slurry and followed by ultrasonic cleaning in ethanol and ultrapure water. Then, in order to self-assemble the capture DNA on electrode, the cleaned GCE was electrodeposited a gold nanoparticles (depAu) layer under the potential of −0.2 V for 30 s. Subsequently, 20 μL of 2.5 μM capture DNA solution was placed on the prepared depAu/GCE surface, and the electrode was kept at room temperature for 16 h. After treatment with 1 mM hexanethiol (HT) for 45 min, the unmodified region of the electrode was blocked and then the resulting HT/capture DNA/depAu/GCE electrode was incubated for 120 min with 20 μL of the OTA aptamer (2.5 μM) solution at room temperature to obtained the OTA aptamer/HT/capture DNA/depAu/GCE electrode. After each step, the electrode was rinsed thoroughly with ultrapure water.

2.3. The detection of OTA and LAMP amplification

The obtained OTA aptamer/HT/capture DNA/depAu/GCE electrode was incubated for 40 min with 20 μL of various concentrations of OTA. Subsequently, the resulting electrode was rinsed with the ultrapure water for the next LAMP reaction. For the LAMP reaction, the electrode was immersed in a total of 1 mL reaction mixture containing 915 μL PBS (pH 7.0, 0.1 M), 70 μL $Ru(phen)_3^{2+}$ (10 mM), 2 μL 10× Thermol Pol Buffer, 1 μL $MgSO_4$ (8 mM), 3 μL betaine (1.0 M), 5 μL dNTPs (1.4 mM), 2 μL of each the FIP and BIP (2.5 μM), 1 μL of backward outer primer B3 (2.5 μM), 8 U of Bst polymerase and 5 μL of DNA template. The LAMP amplification was performed at 65 °C in a calorstat for 60 min and at 80 °C for 4 min to terminate the reaction. Consequently, the reaction mixture of LAMP amplicons was subjected to the ECL analysis with a bare GCE as the working electrode.