Biosensors and Bioelectronics 70 (2015) 268–274

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Electrochemiluminescence biosensor for ultrasensitive determination of ochratoxin A in corn samples based on aptamer and hyperbranched rolling circle amplification

Linlin Yang, Ying Zhang, Ruibao Li, Cuiying Lin*, Longhua Guo, Bin Qiu, Zhenyu Lin*, Guonan Chen

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

ARTICLE INFO

Article history:
Received 25 December 2014
Received in revised form
6 March 2015
Accepted 25 March 2015
Available online 26 March 2015

Keywords:
Ochratoxin A
Aptamer
Electrochemiluminescence
Hyperbranched rolling circle amplification
Corn

ABSTRACT

Determination of ochratoxin A (OTA) is highly important for food safety control. In this study, a signal-on electrochemiluminescence (ECL) biosensor which combined the characteristics of high efficiency of hyperbranched rolling circle amplification (HRCA) and high selectivity of aptamer was developed for OTA determination. The capture probe DNA (CDNA) was firstly immobilized on the gold electrode surface through Au–S interaction, then the OTA aptamer was modified on the electrode surface through hybridization with CDNA. Since OTA can competitively bind with the aptamer due to their high affinity, which would induce the releasing of aptamer from the electrode surface. Subsequently, the free CDNA on the electrode surface can hybridize with the padlock probe and induce HRCA reaction subsequently. Thus, the HRCA products which contained large amount of double-stranded DNA (dsDNA) fragments can be accumulated on the electrode surface. Since $Ru(phen)_3^{2+}$ can intercalate into the groove of dsDNA and acts as ECL indicator, high ECL intensity can be detected from the electrode surface. The enhanced ECL intensity has a linear relationship with OTA in the range of 0.05–500 pg/mL with a correlation coefficient of 0.9957, and the limit of detection (LOD) was 0.02 pg/mL. The developed biosensor has been applied to determine OTA concentration in the corn samples with satisfied results.

1. Introduction

Ochratoxin A (OTA) is a potent toxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum* and which is widely presented in grains and other food products during storage (Scott et al., 1972; Zinedine and Mañes, 2009). Toxicology studies show that OTA has nephrotoxicity, hepatic and immune toxicity, and can cause teratogenic effects to a wide variety of mammalian species (Duarte et al., 2004; Lühe et al., 2003). Currently, the most widely applied methods for OTA detection rely on chromatographic approaches (Tessini et al., 2010) and immunoassays (Meulenberg, 2012). Particularly, thin-layer chromatography (TLC) (Pittet and Royer, 2002), high performance liquid chromatography (HPLC) (Ghali et al., 2008) and enzyme-linked immunosorbent assay (ELISA) (Liu et al., 2008) are recommended by the Association of Official Agricultural Chemists and European Commission. But high cost, long processing times, and the need of specially trained operators have hindered chromatographic approaches' wide application. The enzyme-linked immunosorbent assay (ELISA) owns the advantages of simplicity, reliability and a low requirement for technical skills (Barna-Vetró et al., 1996). However, the preparation of antibodies is time consuming and antibodies may also be susceptible to the problems of stability or modification in the storage and application conditions. Therefore, it is highly demanded to develop new alternatives for OTA determination.

Aptamers are artificial nucleic acids or peptides screened by systematic evolution of ligands by exponential enrichment (SELEX) (Tombelli et al., 2005), which can bind with targets (e.g., proteins, cells, amino acids, organic and inorganic ions) with high affinity and specificity (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Aptamers exhibit significant advantages over antibodies such as easy synthesis, low cost, target versatility and thermal stability under a broad array of conditions. Since Cruz-aguado firstly obtained the aptamer of OTA in 2008 (Cruz-aguado and Penner, 2008), various rapid and sensitive methods for of OTA detection had been reported (Tong et al., 2011; Prabhakar et al., 2011; Castillo et al., 2012; Rhouati et al., 2013).

Electrochemiluminescence (ECL) is a chemiluminescent

* Corresponding authors. Fax: +86 59122866135.
E-mail addresses: lcuiying@fzu.edu.cn (C. Lin), zylin@fzu.edu.cn (Z. Lin).

http://dx.doi.org/10.1016/j.bios.2015.03.067
0956-5663/

reaction triggered by electrochemical methods (Hercules, 1964) and the instrument for ECL detection is simple, cheap and flexible (Cao et al., 2006; Yuan et al., 2011). Moreover, ECL detection owns the character of high sensitivity, and many aptamer based ECL biosensors have been developed in recent years. For example, Yang et al. (2014) proposed a highly sensitive ECL aptasensor for the detection of OTA based on the recovery of the quantum dot (QD) electrochemiluminescence and exonuclease-catalyzed target recycling amplification. The presence of OTA leads to effective removal of the biotin-aptamers from the electrode surface via exonuclease-catalyzed recycling and OTA can be reused for next reaction. Chen et al. (2014) demonstrated a highly sensitive strategy for ECL detection of OTA based on the target-induced autonomous disassembly of the aptamer–DNAzyme supersandwich nanostructures. The presence of the target OTA and the exonuclease resulted in autonomous disassembly of the nanostructures and cyclic reuse of OTA, leading to efficient recovery of the ECL emission and highly sensitive detection of OTA.

Signal amplification technologies, such as polymerase chain reaction (PCR), isothermal strand displacement amplification, rolling circle amplification (RCA), hyperbranched rolling circle amplification (HRCA) and so on, have been integrated into the various detection methods to realize ultrasensitive and trace determination of biotargets related to clinical diagnosis, biomedical research and environmental analysis (Hun et al., 2013; Jiang et al., 2013). Among them, polymerase chain reaction (PCR) is a mature method which has been widely applied in the detection of DNA (Orita et al., 1989; Giordano et al., 2001) and proteins (Zhang et al., 2007). However, PCR needs temperature programmer, sophisticated equipment and trained personnel. These drawbacks have limited its routine use (Nallur et al., 2001; Li et al., 2005; Wang et al., 2005). Compared with PCR, the process of RCA is much simple but the sensitivity of the RCA-based biosensors is poorer than that of HRCA-based biosensors (Zhu et al., 2013). Hyperbranched rolling circle amplification (HRCA) has been developed based on RCA, since 10^9-fold signal amplification process can be accomplished at an isothermal condition (Lizardi et al., 1998), which makes the high sensitivity of HRCA based biosensor. In this type of amplification, the multimeric single-stranded DNA (ssDNA) and dsDNA generated from the initial rolling circle primer extension is amplified by subsequent primer extension, displacement, and ramification (Zhang et al., 2001). HRCA has been applied to develop biosensing systems due to its simplicity, high efficiency, isothermal condition and exponential amplification capacity (Cao and Zhang, 2012; Wang et al., 2013; Cheng et al., 2009).

Herein, a novel ECL biosensor which combined the high selectivity of aptamer, the powerful amplification of HRCA and the high sensitivity of ECL detection has been fabricated for OTA determination. $Ru(phen)_3^{2+}$ molecule which can intercalate into the grooves of dsDNA (Xu et al., 1994) has been chosen as ECL indicator. The enhanced ECL intensity (ΔECL) has a linear relationship with OTA concentration and can be used to represent OTA in the samples. The proposed biosensor had been applied to detect OTA in corn samples with high efficiency. We anticipate that the proposed HRCA based ECL biosensor will find widely applications in food safety control.

2. Experimental section

2.1. Apparatus

ECL intensity versus potential was detected by a laboratory made system, which contains a CHI660D electrochemical workstation (Chenhua Instruments, Shanghai, China) for electrochemical measurements and a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) for ECL emission detection. The photomultiplier tube (PMT) of BPCL ultra-weak luminescence analyzer was operated at −850 V. The experiments were proceeded with a conventional three-electrode system, a 3 mm diameter gold electrode was employed as working electrode, an Ag/AgCl electrode saturated with KCl was used as reference electrode, and a platinum wire served as counter electrode. Cyclic voltammetry (CV) measurement and electrochemical impedance spectroscopy (EIS) characterization were performed in 0.5 M KCl solution containing 0.01 M $[Fe(CN)_6]^{3-/4-}$. ECL analysis was carried out in 0.2 M phosphate buffer solution (PBS) (pH=7.5) containing 0.02 M tripropylamine (TPA) (act as co-react reagent) (Zu and Bard, 2000).

2.2. Reagents and materials

All oligonucleotides used in this research were synthesized by Sangon, Inc., (Shanghai, China) and their sequences are shown as the following:

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′-P–*TAA AGG GAG CAT CGG ACA* TCT TGT ATC CTT TGG TTG AAA CTT CTT CCT TTC TTT CTT C*GA 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′.

The OTA aptamer was complementary to capture probe DNA (CDNA). And the end (in italic) of the 5′-phosphorylated linear padlock probe was complementary to CDNA. In the padlock probe, the binding region for the primer 1 was shown in wavy line, and the region with the same sequence as the primer 2 was shown in underlined portions.

Dichlorotris (1,10-phenanthroline) ruthenium(II) hydrate ($Ru(phen)_3^{2+}$), bovine serum albumin (BSA), tripropylamine (TPA), Tris(2-carboxyethyl) phosphine (TCEP), 6-mercaptohexanol (MCH), OTA, Ochratoxin B (OTB), Ochratoxin C (OTC) and Aflatoxin B1 (AFB1) were purchased from Sigma Aldrich Chemical Co. Ltd. (St. Louis, MO) and used directly without further purification. Deoxynucleotide solution mixture (dNTPs) and nicotinamide adenine dinucleotide (NAD) were purchased from Sangon (Shanghai, China). *Escherichia coli* (*E. coli*) DNA ligase, Bst DNA polymerase large fragment and their corresponding buffer were obtained from New England Biolabs (Beijing, China). The quality control corn samples were purchased from Clover Technology Group, Inc., (Beijing, China). Other chemicals were of analytical reagent grade. All solutions were prepared with deionized water (Milli-Q, Millipore, resistance 18.2 MΩ).

2.3. Electrode preparation and ECL biosensor fabrication

The gold electrode (3 mm in diameter) was polished with 1.0, 0.3 and 0.05 μm alumina slurry orderly to obtain a mirror surface and then sonicated in ethanol and deionized water for 3 min, respectively. After drying under nitrogen gas, the gold electrode was subjected to cyclic potential scanning between −0.2 and 1.6 V in 0.5 M H_2SO_4 solution until a typical stable voltammetric peak was obtained. Further, the gold electrode was thoroughly rinsed with deionized water and dried with nitrogen gas. Next, the gold electrode was immersed in a thiolated CDNA (1 μM) solution at room temperature for 2 h in dark to modify CDNA on the gold electrode surface through Au–S interaction, and then the unoccupied sites were blocked with 1 mM MCH. Then the CDNA modified electrode was immersed in the hybridization buffer which contained 1 μM aptamer, 50 mM Tris–HCl (pH 7.4), 150 mM

NaCl, 1 mM $MgCl_2$ and 10 mM KCl and kept at 37 °C for 4 h to make aptamer hybridize with CDNA. The resulting CNDA/aptamer electrode was then washed with deionized water to remove unhybridized aptamers.

The prepared CNDA/aptamer electrode was soaked in the solution containing various concentrations of OTA at 37 °C for 1 h. Then the modified electrode was immersed in the solution which contained 50 nM padlock probe, 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$ and 10 mM KCl and kept at 37 °C for 1 h. Subsequently, the ligation reaction was incubated at 37 °C for 1 h in the ligation buffer solution consisting of 6 U *E. coli* DNA ligase, 0.05% BSA and 0.167 mM NAD. Next, the HRCA-reaction was proceeded at 63 °C for 80 min by immersing the achieved electrode in 150 μL HRCA-reaction buffer, which containing 1 μM primer 1, 1 μM primer 2, 8 U Bst DNA polymerase, and 0.6 mM dNTPs. After this, the electrode was washed with Tris–HCl solution to inactivate the enzyme.

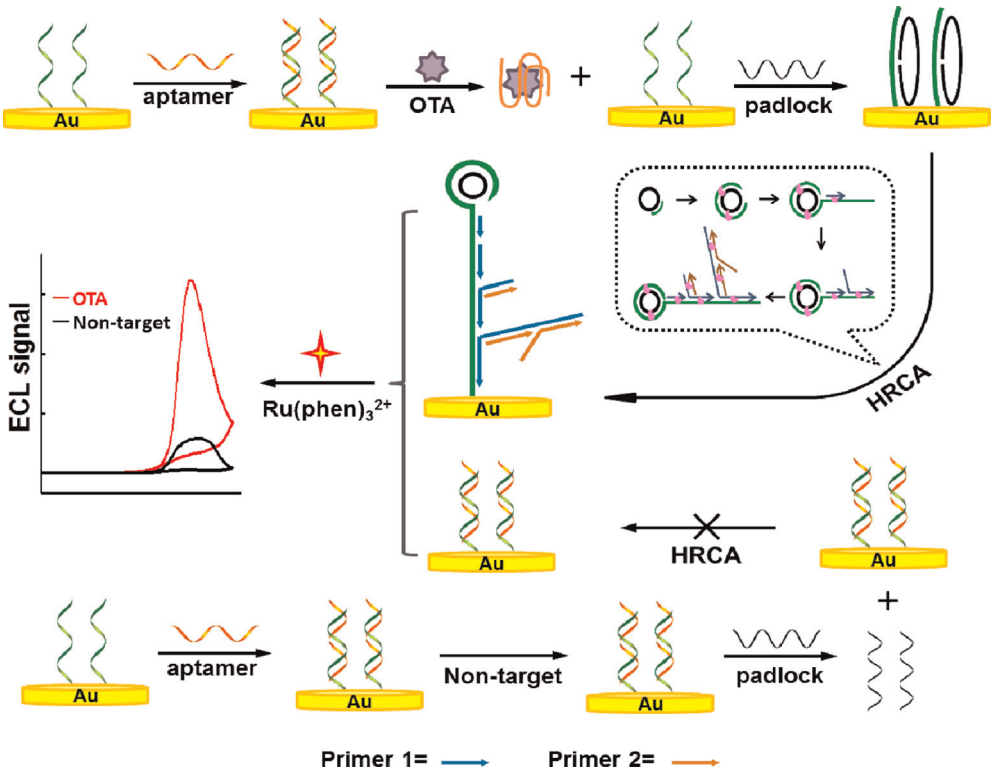
2.4. ECL determination

The achieved electrode was immersed in 1 mM $Ru(phen)_3^{2+}$ solution at 4 °C for 5 h to make $Ru(phen)_3^{2+}$ molecules intercalate into dsDNA grooves. Then the electrode has been rinsed thoroughly with PBS (0.2 M) to reduce the nonspecific binding. Finally, the ECL signal from the modified electrode was measured in the potential range of 0.6–1.5 V versus Ag/AgCl in 0.2 M PBS buffer containing 0.02 M TPA, the scan rate was settled at 100 mV/s. Each sample was repeated for 3 times and the averaged value was adopted as the reference result. The error bars show the standard deviation of three replicate determinations.

3. Results and discussion

3.1. Principle of the ECL biosensor

The principle of the proposed ECL biosensor is shown in Scheme 1. The CDNA is firstly immobilized on the gold electrode surface via Au–S covalent bonds, then the OTA aptamer is modified on the electrode surface through hybridization with CDNA. In the presence of OTA, the aptamer prefers to form OTA–aptamer complex in lieu of the aptamer–CDNA duplex, leading to the releasing of aptamer from the electrode surface and make CDNA free on the electrode surface again. Subsequently, the free CDNA can hybridize with the italic part of the padlock probe, and the padlock probe is ligated and circularized with the action of *E. coli* DNA ligase following. Afterwards, the HRCA reaction proceeded on the electrode surface. Briefly, primer 1 is extended isothermally at its 3′ end by Bst DNA polymerase to generate multimeric ssDNA, which serves as the template for the primer 2 binding. Primer 2 is further extended by Bst DNA polymerase to displace the downstream growing DNA strands. This displaced strand contains multiple binding sites for primer 1 hybridization again (Zhu et al., 2013; Wang et al., 2013). Consequently, the HRCA products which contained a large number of dsDNA fragments with different lengths are accumulated on the electrode surface. Then $Ru(phen)_3^{2+}$ was intercalated into the dsDNA grooves (Chen et al., 2012) and act as ECL indicator, which results in the enhancement of the ECL intensity from the electrode surface. In the absence of OTA, aptamer cannot separate from aptamer–CDNA duplex, so the CDNA cannot combine with padlock probe, and HRCA is not initiated on the electrode surface. So only weak ECL signal can be detected. The enhanced ECL intensity has a direct relationship with the OTA concentration and a sensitive biosensor for OTA can be developed based on which.



Scheme 1. The principle of the HRCA based ECL biosensor for OTA.

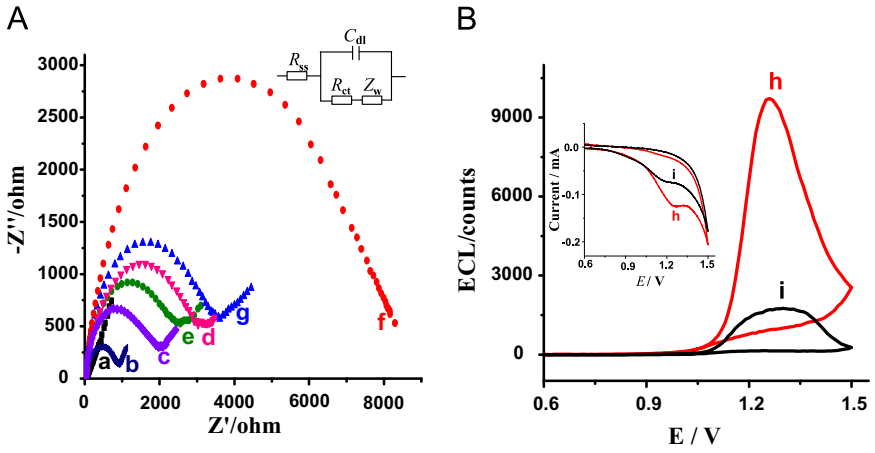


Fig. 1. (A) Nyquist plots corresponding to different electrodes. (a) bare Au electrode; (b) CDNA modified electrode; (c) MCH/CDNA modified electrode; (d) CDNA/aptamer modified electrode; (e) react with 5 pg/mL OTA; (f) execute the HRCA in the presence of 5 pg/mL OTA; (g) execute the HRCA in the absence of OTA. (B) ECL signal of the developed sensing system in the presence (h) and absence (i) of 5 pg/mL OTA (the inset shows the corresponding CV curves).

3.2. Characterization of the modified electrodes

EIS had been applied to monitor the electrode modification. The equivalent circuit as the model of the working electrode has been adopted to fit EIS data (shown in the up-right corner of Fig. 1 (A)), where C_{dl} is the double-layer capacitance, R_{ct} is the surface charge transfer resistance, Z_w is the Warburg element, and R_s is the resistance of the electrolyte solution and all the connections. In the impedance spectra, the semicircle diameter of impedance equals the electron transfer resistance (R_{ct}). As shown in Fig. 1(A), the bare Au electrode (curve a) shows a very small semicircular domain (R_{ct}), this indicated that the electro active ions transported easily on the electrode surface. Causing by the electrostatic repulsion between the negatively charged phosphate backbone of the CDNA and the anionic $[Fe(CN)_6]^{3-/4-}$ ions, the R_{ct} increased to about 1000 Ω after the modification of thiolated CDNA (curve b). After the blocking of the CDNA modified electrode with MCH, the R_{ct} was increased significantly (2000 Ω, curve c) due to the hindrance on the electrode. The hybridization of aptamer with CDNA on the electrode surface resulting the further increasing of R_{ct} (3000 Ω, curve d), which can be explained that the double negative charged phosphate backbones on the electrode surface and the longer electron-transfer distance further prevent $[Fe(CN)_6]^{3-/4-}$ charge transfer on the electrode interface. However, after reaction with OTA (5 pg/mL), the R_{ct} decreased to 2600 Ω (curve e), the reason maybe lie in that the aptamer has been released from the electrode surface. And after the HRCA reaction, the R_{ct} value was sharply increased to about 8000 Ω (curve f), this can be explained by the fact that the HRCA products contained large amount of dsDNA had been accumulated on the electrode surface. In the absence of OTA, and the other conditions being the same as HRCA conditions, the R_{ct} value was only 3500 Ω (curve g), a little bigger than that of curve d. The reason lies in that there has a small amount of nonhybridized CDNA on the electrode surface and which can hybridize with the padlock probe and induce HRCA reaction, so a slightly increasing of the R_{ct} value can be reached. Fig. 1(B) shows the CV curves (inset figure) and the corresponding ECL signal in the presence and absence of OTA (5 pg/mL). In the absence of OTA, only a weak anodic current and a weak ECL signal had been recorded (curve i), but a large increase in the anodic current of $Ru(phen)_3^{2+}$ oxidation and a strong ECL signal had been detected in the presence of the traget (curve h). These phenomena also indicated that a large amount of dsDNA fragments generated from HRCA reaction accumulated on the electrode surface and $Ru(phen)_3^{2+}$ intercalated into dsDNA grooves to act as the ECL probe. These results indicated the credible of our proposed biosensor.

3.3. Optimization of the reaction conditions

The parameters which affect the performance of the biosensor had been optimized. *E. coli* DNA ligase plays a decisive role in the ligation and circularization of the padlock probe, and Bst DNA polymerase is a key factor for primer extension and strand displacement, so their dosages had been optimized firstly. As shown in Fig. 2(A), the ECL signal increased with increasing dosage of *E. coli* DNA ligase from 2 U to 6 U and then reached saturations after 6 U, this indicated that 6 U *E. coli* DNA ligase was sufficient for the ligation reaction of padlock probes. As for Bst DNA polymerase, the ECL signal increased with increasing dosage from 3.2 U to 9.6 U, this suggested as more Bst DNA polymerase was added, more HRCA products were produced, and beyond 9.6 U, the ECL signal did not exhibit further increasing which due to 9.6 U Bst DNA polymerase was enough for HRCA reaction. Thus 6 U *E. coli* DNA ligase and 9.6 U Bst DNA polymerase were chosen as the optimal dosage during the following studies. dNTPs is another key factor for strand extension, so the concentration of dNTPs was optimized too. The ECL intensity increased gradually with the increasing concentration of dNTPs from 0.2 mM to 0.6 mM, and then the ECL intensity reached a plateau without any increasing when the concentration of dNTPs was above 0.6 mM (Fig. 2(B)). This suggested 0.6 mM was sufficient for HRCA reaction; this may be due to the spatial steric effect of the HRCA products at higher concentration and the limited surface area of the electrode, which would hinder the further HRCA reaction. Therefore, 0.6 mM of dNTPs was chosen for the subsequent experiments.

Since the reaction time of HRCA directly affected the amount of HRCA products, it is essential to optimize the reaction time of HRCA for improvement of performance of the biosensor. As shown in Fig. 2 (C), the ECL intensity of the system increased firstly with the increasing of the HRCA reaction time and tended to level off at about 80 min. This indicated that 80 min was enough for the reaction to completion, considering the fabrication efficiency, 80 min was adopted in the following study. Furthermore, the incubation time for intercalation of $Ru(phen)_3^{2+}$ on the dsDNA accumulated on the electrode surface was optimized too (shown in Fig. 2(D)). The ECL intensity increased gradually with the increasing of the intercalation time from 1 h to 5 h, and then reached a stabilized platform when the intercalation time exceeded 5 h. Hence, 5 h was selected as the optimal intercalation time throughout the experiments.

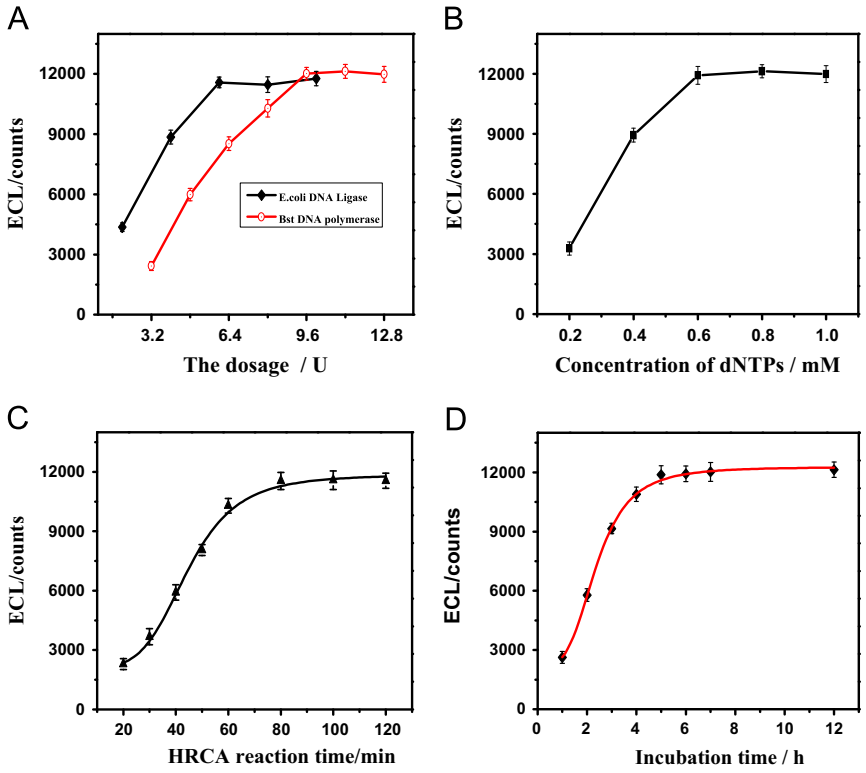


Fig. 2. Optimization of the reaction conditions. (A) Effect of the dosage of *E. coli* DNA ligase and Bst DNA ploymerase on the ECL intensity with 50 pg/mL OTA. (B) Effect of dNTPs concentration on ECL intensity with 50 pg/mL OTA. (C) Effect of the HRCA reaction time on the ECL intensity. (D) Effect of intercalation time between $Ru(phen)_3^{2+}$ and dsDNA on the ECL intensity. The error bars show the standard deviation of three replicate determinations.

Another important reaction factor was pH since which can affect the activities of *E. coli* DNA ligase and Bst DNA polymerase. The influence of reaction pH values from 2 to 10 were investigated, the results showed that the ECL intensities were weak if pH values were lower than 5 or higher than 8, but the ECL intensities have no substantial differences under pH values from 5 to 8 (data not shown). The possible reason for this phenomenon could be attributed to *E. coli* DNA ligase or Bst DNA polymerase were inactivated in strong acid and strong alkali environment. Based on physiological conditions, pH 7.4 was adopted as the optimized condition.

3.4. Calibration curve for OTA determination

Fig. 3(A) shows the ECL responses after reaction with different concentrations of OTA had been recorded under optimized conditions. ECL signal increased gradually with the increasing of OTA concentration in the range of 50 fg/mL to 100 ng/mL. Fig. 3 (B) shows the relationship between the enhanced ECL intensity (ΔECL, the difference ECL intensity between the presence and absence of OTA) and the OTA concentration. And there has a linear relationship between ΔECL and the logarithm of OTA concentration from 0.05 pg/mL to 500 pg/mL with a correlation coefficient of 0.9957. The linear equation was ΔECL/counts = 6107.6 + 2371 log C_{OTA}. The detection limit (LOD) was 0.02 pg/mL (calculated according to the signal which was equivalent to 3 times the standard deviation of the blanks).

The performance of the proposed biosensor was compared with the other aptamer-based sensors reported in literatures for the detection of OTA. Apparently, the proposed ECL aptasensor had a lower detection limit for OTA than RCA-based electrochemical sensors (Tong et al., 2012; Huang et al., 2013), nanomaterial-based

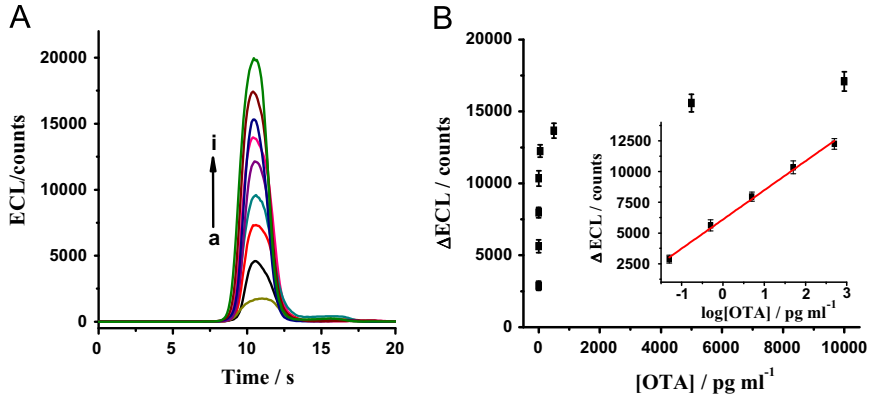


Fig. 3. (A) ECL response at different OTA concentrations. (a–i) 0, 50 fg/mL, 500 fg/mL, 5 pg/mL, 50 pg/mL, 500 pg/mL, 5 ng/mL, 50 ng/mL, and 100 ng/mL. (B) The relationship between ΔECL and the OTA concentrations. The inset shows the calibration curve between the logarithm of OTA concentrations and ΔECL. The error bars show the standard deviation of three replicate determinations.

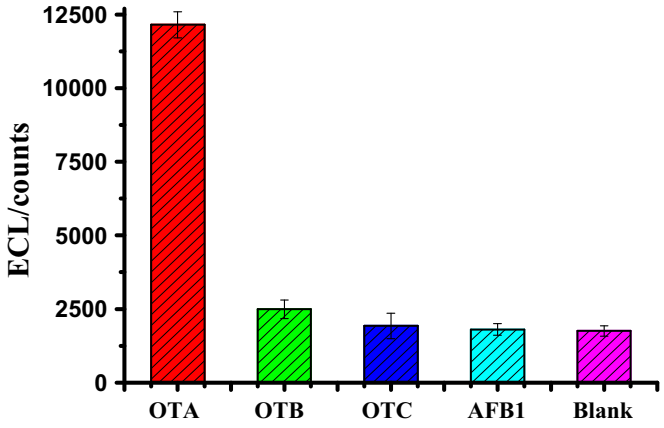


Fig. 4. The selectivity the developed ECL biosensor to the target. The error bars show the standard deviation of three replicate determinations.

Table 1
Detection results of OTA in corn samples using the proposed ECL aptasensor ($n=5$).

Sample	Detected (ng/g)	Reference (ng/g)	Spiked (ng/g)	Total found (ng/g)	Recovery (%)	RSD(%)
1	53.12	54.20	20.00	71.40	91.4	4.7
2	55.76	54.20	50.00	105.01	98.5	5.3
3	53.57	54.20	100.00	149.17	95.6	4.4

sensors (Jiang et al., 2014; Kuang et al., 2010; Chen et al., 2014) and exonuclease-catalyzed ECL sensor (Yang et al., 2014), which can be attributed to the high efficiency of HRCA.

3.5. Selectivity, reproducibility and stability of the ECL biosensor

The selectivity of the fabricated ECL biosensor was studied also. OTB, OTC and AFB1, which were at the 100-fold concentrations of the target (50 pg/mL OTA) had been chosen as the interferents. Compared with that from the control system without target OTA, the ECL intensities from OTB, OTC and AFB1 showed no obvious variations, while OTA induced a greatly ECL enhancement (shown in Fig. 4). These results indicate that the proposed biosensor has good selectivity to the target.

The fabrication reproducibility was carried out by comparing the ECL intensity from five different ECL biosensors when the concentration of the target was settled at 50 pg/mL. The relative standard deviation (RSD) was 6.7%, revealing that this method had good reproducibility. We also tested the stability of the proposed biosensor by detecting OTA (5 pg/mL) with the fabricated ECL biosensor, the modified electrode had been stored at 4 °C for 10 days, and only 3% decreases in the ECL intensity had been detected compared with the fresh prepared one, this indicated the good stability of the developed aptasensor.

3.6. Determination of OTA in corn samples

The accuracy of the proposed biosensor was evaluated by testing the OTA in the quality control corn samples. The samples were extracted by 80% methanol solution firstly the concentrations of OTA in the corn samples were detected to be 53.12 ng/g, 55.76 ng/g and 53.57 ng/g, respectively, nearly the same as the reference concentrations. Then the recoveries were examined by the standard addition method, 20.00 ng/g, 50.00 ng/g and 100.00 ng/g OTA standard solution had been spiked in the above mentioned three samples respectively, as shown in Table 1, the recoveries were in the range 91.4–98.5%. These results indicated that the prepared ECL biosensor was effective and reliable for the analysis of real samples.

4. Conclusion

In summary, a reliable and highly sensitive HRCA-based ECL biosensor which combined the advantages of high selectivity of aptamer, high amplification efficiency of HRCA and high sensitivity of ECL technique had been developed for OTA determination. The LOD of the proposed biosensor is 0.02 pg/mL. The sensor had been applied to detect OTA in the corn samples with satisfied results.

Acknowledgment

This project was partly financially supported by National Natural Science Foundation of China (21175024, 21222506, 21275031), program for New Century Excellent Talents in University (NCET-12-0619), and the Natural Science Foundation of Fujian Province (2014J06005).

References

Barna-Vetró, I., Solti, L., Téren, J., Gyöngyösi, Á., Szabó, E., Wölfling, A., 1996. J. Agric. Food Chem. 44 (12), 4071–4074.
Cao, A.P., Zhang, C.Y., 2012. Anal. Chem. 84 (14), 6199–6205.
Cao, W., Ferrance, J.P., Demas, J., Landers, J.P., 2006. J. Am. Chem. Soc. 128 (23), 7572–7578.
Castillo, G., Lamberti, I., Mosiello, L., Hianik, T., 2012. Electroanalysis 24 (3), 512–520.
Chen, Y., Yang, M.L., Xiang, Y., Yuan, R., Chai, Y.Q., 2014. Nanoscale 6 (2), 1099–1104.
Chen, Y., Xu, J., Su, J., Xiang, Y., Yuan, R., Chai, Y.Q., 2012. Anal. Chem. 84 (18), 7750–7755.
Cheng, Y.Q., Zhang, X., Li, Z.P., Jiao, X.X., Wang, Y.C., Zhang, Y.L., 2009. Angew. Chem. Int. Ed. 48 (18), 3268–3272.
Cruz-aguado, J.A., Penner, G., 2008. J. Agric. Food Chem. 56 (22), 10456–10461.
Duarte, S.C., Pena, A., Lino, C.M., 2004. Food Microbiol. 27 (2), 187–198.
Ellington, A.D., Szostak, J.W., 1990. Nature 346 (6287), 818–822.
Ghali, R., Hmaissia-Khlifa, K., Mezigh, C., Ghorbel, H., Maaroufi, K., Maschgoul, S., Hedilli, A., 2008. Anal. Lett. 41 (5), 757–766.
Giordano, B.C., Ferrance, J., Swedberg, S., Hühmer, A.F.R., Landers, J.P., 2001. Anal. Biochem. 291 (1), 124–132.
Hercules, D.M., 1964. Science 145 (3634), 808–809.
Huang, L., Wu, J.J., Zheng, L., Qian, H.S., Xue, F., Wu, Y.C., Pan, D.D., Adeloju, S.B., Chen, W., 2013. Anal. Chem. 85 (22), 10842–10849.
Hun, X., Liu, F., Mei, Z.H., Ma, L.F., Wang, Z.P., Luo, X.L., 2013. Biosens. Bioelectron. 39 (1), 145–151.
Jiang, L., Qian, J., Yang, X.W., Yan, Y.T., Liu, Q., Wang, K., Wang, K., 2014. Anal. Chim. Acta 806, 128–135.
Jiang, L.P., Peng, J.D., Yuan, R., Chai, Y.Q., Yuan, Y.L., Bai, L.J., Wang, Y., 2013. Analyst 138 (17), 4818–4822.
Kuang, H., Chen, W., Xu, D.H., Xu, L.G., Zhu, Y.Y., Liu, L.Q., Chu, H.Q., Peng, C.F., Xu, C. L., Zhu, S.F., 2010. Biosens. Bioelectron. 26 (2), 710–716.
Li, F., Zhao, C., Zhang, W., Cui, S., Meng, J., Wu, J., Zhang, D.Y., 2005. J. Clin. Microbiol. 43 (12), 6086–6090.
Liu, B.H., Tsao, Z.J., Wang, J.J., Yu, F.Y., 2008. Anal. Chem. 80 (18), 7029–7035.
Lizardi, P.M., Huang, X., Zhu, Z., Bray-Ward, P., Thomas, D.C., Ward, D.C., 1998. Nat. Genet. 19 (3), 225–232.
Lühe, A., Hildebrand, H., Bach, U., Dingermann, T., Ahr, H.J., 2003. Toxicol. Sci. 73 (2), 315–332.
Meulenberg, E.P., 2012. Toxins 4 (4), 244–266.
Nallur, G., Luo, C., Fang, L., Cooley, S., Dave, V., Lambert, J., Kukanskis, K., Kingsmore, S., Lasken, R., Schweitzer, B., 2001. Nucleic Acids Res. 29 (23), E118.
Orita, M., Suzuki, Y., Sekiya, T., Hayashi, K., 1989. Genomics 5 (4), 874–879.
Pittet, A., Royer, D., 2002. J. Agric. Food Chem. 50 (2), 243–247.
Prabhakar, N., Matharu, Z., Malhotra, B.D., 2011. Biosens. Bioelectron. 26 (10), 4006–4011.
Rhouati, A., Yang, C., Hayat, A., Marty, J.L., 2013. Toxins 5 (11), 1988–2008.
Scott, P.M., Van-Walbeek, V., Anyeti, O., 1972. J. Agric. Food Chem. 20 (6), 1103–1109.
Tessini, C., Mardones, C., Von Baer, D., Vega, M., Herlitz, E., Saelzer, R., Silva, J., Torres, O., 2010. Anal. Chim. Acta 660 (1), 119–126.
Tombelli, S., Minunni, M., Mascini, M., 2005. Biosens. Bioelectron. 20 (12), 2424–2434.
Tong, P., Zhang, L., Xu, J.J., Chen, H.Y., 2011. Biosens. Bioelectron. 29 (1), 97–101.
Tong, P., Zhao, W.W., Zhang, L., Xu, J.J., Chen, H.Y., 2012. Biosens. Bioelectron. 33 (1), 146–151.

Tuerk, C., Gold, L., 1990. Science 249 (4968), 505–510.
Wang, B., Potter, S.J., Lin, Y., Cunningham, A.L., Dwyer, D.E., Su, Y., Ma, X., Hou, Y., Saksena, N.K., 2005. J. Clin. Microbiol. 43 (5), 2339–2344.
Wang, Q.P., Zheng, H.Y., Gao, X.Y., Lin, Z.Y., Chen, G.N., 2013. Chem. Commun. 49 (97), 11418–11420.
Xu, X.H., Yang, H.C., Mallouk, T.E., Bard, A.J., 1994. J. Am. Chem. Soc. 116 (18), 8386–8387.
Yang, M.L., Jiang, B.Y., Xie, J.Q., Xiang, Y., Yuan, R., Chai, Y.Q., 2014. Talanta 125, 45–50.
Yuan, T., Liu, Z.Y., Hu, L.Z., Xu, G.B., 2011. Chin. J. Anal. Chem. 39 (7), 972–977.
Zhang, D.Y., Zhang, W., Li, X., Konomi, Y., 2001. Gene 274 (1), 209–216.
Zhang, H., Zhao, Q., Li, X.F., Le, X.C., 2007. Analyst 132 (8), 724–737.
Zhu, X., Xu, H.F., Zheng, H.Y., Yang, G.D., Lin, Z.Y., Qiu, B., Guo, L.H., Chi, Y.W., Chen, G. N., 2013. Chem. Commun. 49 (86), 10115–10117.
Zinedine, A., Mañes, J., 2009. Food Control 20 (4), 334–344.
Zu, Y.B., Bard, A.J., 2000. Anal. Chem. 72 (14), 3223–3232.