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Facile combination of beta-cyclodextrin host-guest recognition with exonuclease-assistant signal amplification for sensitive electrochemical assay of ochratoxin A

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ABSTRACT

Smartly coupling exonuclease-induced target recycling signal amplifications with β-cyclodextrin host-guest recognition, a novel "signal-on" aptamer sensor for sensitive determination of ochratoxin A (OTA) was proposed for the first time. Firstly, the formation of double-strand DNA (dsDNA) was occurred by hybridizing OTA aptamer with its complementary DNA (cDNA) and as the probe DNA the cDNA at its 3′ terminal was labeled with methylene blue (MB). Next, when OTA was present, the aptamer tended to form aptamer-OTA complex with conformation of G-quadruplex instead of aptamer-cDNA duplex, leading to thus the probe DNA separating from dsDNA complex. Then the RecJf exonuclease was added, demolishing partially G-quadruplex structure and releasing a certain number of OTA. Sequentially, those released OTA would continue to react with the rest of aptamer in dsDNA, drawn into development of a new round of G-quadruplex complex, where the target cycling was realized. Meanwhile, as a signal molecule, MB modified on cDNA was liberated along with the cDNA being digested into monoucleotides by RecJf exonuclease, capable of diffusing onto the electrode surface due to host-guest recognition with β-cyclodextrin, whereupon the signal was enriched and yielded. In this way, cycles of target with continuous output of signal indicators were undergone, in which the detection of target was in return fulfilled with signal amplification owing to the joint endeavor of exonuclease and β-cyclodextrin. Under the optimal conditions, the raising signal maintained a linear relation with the logarithm of the target concentrations ranging from 10 pg/mL to 10.0 ng/mL and the detection limit reached as low as 3 pg/mL. This brand-new strategy was simple and low-cost but satisfactory in terms of detection limit, range and sensitivity, in all possibility to be applied extensively for diverse targets detection by easily alternating the corresponding aptamers.

1. Introduction

Given its hazardous nature of carcinogenicity, nephrotoxicity and immunuetoxicity, ochratoxin A (OTA) is recently an interest for research pursued by diverse fields worldwide (Mayura et al., 1984; Assaf et al., 2004). To track its origin, as one of mycotoxins, OTA was a toxic secondary metabolite commonly secreted by fungi Aspergillus and Penicillium, presenting ubiquitously in a variety of foodstuffs involving rice, peanut, wheat, maize and ultimately finding its way to the human body through food chain (Wang et al., 2018; Marin et al., 2013). With apprehension for the risks this bugbear might bring to human health, authorities exerted a relatively strict control over OTA. For instance, International Agency for Research on Cancer proclaimed its possible carcinogenicity towards humans; the European Commission raised the threshold for regulating a maximized amount of OTA as low as 5 μg/kg from raw cereal grains (Pfohl-Leszkowicz et al., 2007; Dall'Asta et al., 2010). Nevertheless, appreciable uncertainties were arisen presumably from robust chemical stability of OTA which enabled its survival from the modern food process under even severe physicochemical conditions. Worse, once ingested by human, it was metabolized quite slowly that could exist in the body for over one month, adversely affecting human health for an extended period. Therefore, the surging practical

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need for accurately monitoring the level of OTA is calling admitting of no delay for imperative developments in superior strategies on its assay with well-performed sensitivity and specificity.

Various analytical methods were hitherto employed for OTA quantification, such as mass spectrometry (De Santis et al., 2017; Ventura et al., 2003), high-performance liquid chromatography (Asadi, 2018), gas chromatography (Soleas et al., 2001) enzyme-linked immunosorbent assay (Garcia-Fonseca et al., 2016), capillary electrophoresis (Lee et al., 2013), surface enhanced Raman scattering (Li et al., 2018a), electrochemiluminescence (Zhang et al., 2018; Wang et al., 2016a), fluorescence assay (Wang et al., 2017, 2015; Ma et al., 2018) and colorimetric assay (Lin et al., 2018; Yu et al., 2018). Admittedly, these reported methods made undeniable progress otherwise compromised somewhat by unavoidable deficiencies of expensive equipment, time-consuming, unsatisfying sensitivity, and complicated process and demanding expert operators. To be noted however, electrochemical sensors became prevalent as an intriguing approach for biosensing and garnered considerable attention by virtue of desirable sensitivity, specificity, rapidity and affordability for instruments, operators-friendly process and miniaturization of the instruments (Madhurantakam et al., 2018; Abi et al., 2018; Li et al., 2018b; Li et al., 2018c). In particular, when associated with a single stranded oligonucleotide sequence-aptamer who was capable of binding with versatile targets with impressive affinity and specificity, the aptasensor raised considerable concerns about assaying mycotoxins attributed to the exceptional merits of outstanding binding constants for the majority of mycotoxins along with dissociation constants in the scope of nanomole (Zhu et al., 2018; Jiang et al., 2018; Shi et al., 2018).

Target recycling assisted by enzymes offered an appealing alternative for fulfillment of signal amplification. As a specific biocatalyst enzyme, nucleases could initiate signal amplification, in which a single target would result in appreciable cycles of target, effectively recognizing and sensitively cleaving the phosphodiester bonds both from the ends and internally between nucleotides of nucleic acids (Chen et al., 2018; Yang et al., 2018; Cui et al., 2018). It is of superiority over many other amplification strategies catalyzed by substances as nanoparticles, graphene and quantum dot due mainly to its simplified process without demanding complicated preparation, thus enjoying wide employment in signal amplification (Wang et al., 2016b; Jiang et al., 2014; Chu et al., 2016). Previously, signal amplification strategies in this kind were reported utilizing exonucleases. To name a few, Zhao et al. established an OTA detection platform based on signal amplification by exonuclease III and fluorescence quenching by gold nanoparticles (Zhao et al., 2018). Ni et al. developed a label-free electrochemiluminescence aptasensor using exonuclease-assisted target recycling amplification for the detection of OTA (Ni et al., 2018). More interestingly, as a specific exonuclease acting exclusively on ssDNA, RecJf exonuclease bore the capability of catalyzing the deoxy-nucleotide monophosphates's removal from ssDNA in the direction of 5′ → 3′, even including those aptamers bound with target in a complex of target-aptamer, which turned themselves to be a favorite introduced to various novel electrochemical aptasensors strategy.

Inspired by as-mentioned items, in this study, using exonuclease-catalyzed target recycling signal amplification and β-cyclodextrin host-guest recognition, an OTA aptasensor on the basis of electrochemistry was thus fabricated. At first, the hybridization was done between OTA aptamer and its complementary DNA (probe DNA) modified with MB as signal indicator for formation of dsDNA. Then OTA was added binding with aptamer to shape OTA-aptamer complex with structure of G-quadruplex, which dissociated the hybridized cDNA from dsDNA simultaneously. Afterwards, RecJf exonuclease was added, functioning as a catalyst, destroying certain amounts of OTA-aptamer complexes and releasing the OTA who would engage in another loop of G-quadruplex formation. With the same pace of above cycling, cDNA would be digested into mononucleotides by the exonuclease, releasing the signal molecules that were capable of diffusing easily onto the electrode surface through host-guest recognition with β-cyclodextrin, and contributing accordingly to the enrichment and production of the signal revealed by differential pulse voltammetry (DPV). Hence the quantification of OTA was expected to be accomplished by recording the signal variances on electrode surface before and after the addition of OTA. To be noted, two tricks would be employed for the realization of pre-eminence performance by this proposed assay: for one hand, exonuclease was adopted to resect both OTA-aptamer and cDNA into fragments, resulting in the former's release of OTA involved in reaction cycles, and the latter's continuous liberation of MB with the same pace of the cycle served as the signal molecules accessible to the electrode surface where the signal was produced and amplified. For the other hand, β-cyclodextrin modified on the electrode surface could facilitate significantly the signal molecule enrichment via the host-guest interaction. In this way, a versatile and sensitive exonuclease-activated target recycling electrochemical tactic was presented which was unprecedentedly reported integrating all these novel elements to the best of our knowledge, holding thus a profound potential to be extensively applied for the quantification of the rest of targets after conveniently replacing the corresponding aptamer.

2. Experimental section

2.1. Reagents and materials

The OTA (10 μg/mL in acetonitrile, analytical standard, Sigma-Aldrich), mercapto-β-cyclodextrin (β-CD-SH), aflatoxin B1 (AFB1), ochratoxin B (OTB), aflatoxin M1 (AFM1), ochratoxin C (OTC) were all purchased from Sigma-Aldrich (Sigma-Aldrich, China) and utilized without treatment. 10 × NEBuffer 2 (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH = 7.9) and RecJf exonuclease were bought in New England Biolabs Ltd. (Beijing, China). The remnant reagents were all in analytical grade directly utilized as purchased with no treatments. As the solvent, the ultrapure water was exploited with which the solutions were prepared and diluted from the Millipore Milli-Q system (Millipore, Billerica, MA, 18.2 MΩ cm).

The cDNA sequences and aptamer of OTA were all synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). MB for the purpose of realizing the signal transmission was modified on 3′ end of cDNA. The oligonucleotide sequences of aptamer and cDNA were exhibited below:

Aptamer: 5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3′
cDNA: 5′- TGT CCG ATG CTC CCT TTA CGC CAC CCA CAC CCG ATC- MB-3′

2.2. Pretreatment of electrode

A portable screen-printed electrode (SPE, DRP-C220AT, Metrohm China Limited) was utilized comprising an Ag reference electrode, a gold working electrode, as well as a gold counter electrode. Prior to treatment with pure water for about 10 min for removal of residual organic contaminants, for another 10 min the electrodes were foremost cleaned in an ultrasonicator with ethanol. Sequentially, the electrodes were rinsed with sufficient amount of pure water, blew till dried by N_2. At room temperature every electrochemical measurement was implemented with a CHI660E electrochemical workstation (Shanghai Chenhua Instrument Corporation, China). It was also at room temperature to steer DPV in sensor interrogation. The potentials were with reference to Ag reference electrode. Each step was operated by independent experiments repeating for no less than five times.

2.3. RecJf exonuclease-assisted target recycling reaction

Before usage, every oligonucleotide was heated for 5 min at 95 °C,

cooled down to room temperature for upcoming experiments, ensuring the aptamer and cDNA to adjust to their inherent conformations. The occurrence of hybridization between the aptamer and cDNA was in 200 μL of 1 × NEBuffer 2, experiencing identically the process of annealing. After above process, a range of OTA concentrations as well as 2 μL RecJf exonuclease (1 U/μL) were dropped into the solution and then incubated for 90 min at 37 °C for adequate reaction. AFB1 (10 ng/mL), AFM1 (10 ng/mL), OTC (10 ng/mL) and OTB (10 ng/mL) were picked out to displace OTA in the specificity test.

2.4. Gel electrophoresis

Putting 0.75 g of agarose powder into TAE buffer (40 mM Tris-Acetic Acid; 2 mM EDTA), a 2% agarose gel was thus made. Heat was used for the dissolution of the power into the mixed solution. Sequentially, the gel was poured into the gel setting tray. Eventually, 5 μL aptamer, MB-cDNA, dsDNA, dsDNA + RecJf exonuclease, aptamer + RecJf exonuclease, dsDNA + RecJf exonuclease without and with OTA mixed respectively with 1 μL of DNA loading dye to be electrophoresed for 80 min at 120 V. The results of electrophoretogram were observed and photographed by a Gel Doc XR System (BIO-RAD, USA).

2.5. Determination of OTA

First of all, at room temperature, 5.0 μg/mL β-CD-SH was added onto the electrode surface and then incubated for 120 min. Next, through rinsing with PBS buffer for 3 times, the needless β-CD-SH was eliminated from the electrode surface. Based on the β-CD/SPE, at last the above mentioned reaction solution after addition of RecJf exonuclease was designated to determine target at various concentrations under optimal circumstance. Sequentially 10 μL reaction solutions were dropped onto the β-CD/SPE's surface. After capturing for 60 min, the electrode rinsed with PBS buffer before electrochemical measurements.

2.6. Selectivity

Employing analogs in the sensing platform, the specificity of the as-proposed strategy was assessed. Four different interfering mycotoxins as representatives-AFM1 and AFB1, OTB, OTC were appointed respectively for examining the selectivity of the strategy at the concentration of 10 ng/mL, going through the same experimental process as the one illustrated for the OTA detection. The results were compared with the one using 1.0 ng/mL OTA.

2.7. Determination of OTA in real samples

Recovery was validated in red wine samples using OTA at various concentrations. The possibility and reliability towards the method being applied in practice were established in regard of evaluating the recovery rate in actual samples. At standard samples (1.0, 2.0 and 5.0 ng/mL), the red wine samples were spiked with a range of concentrations of OTA. The procedures were identical with the above one elaborated.

3. Results and discussions

3.1. Sensing principle of the aptasensor for OTA detection

The principle of this proposed strategy was exhibited in Fig. 1: firstly, in homogeneous solution, the aptamer of target and its cDNA (probe DNA) were hybridized to develop dsDNA, whereas the MB as signal indicator was modified on cDNA. At this stage, very limited amount of dsDNA could diffuse onto the surface of the electrode because of the steric-hindrance effect between the dsDNA complex and the β-cyclodextrin modified electrode, thereby leading to limited variation of the DPV signal. Whereas when the target presented, the aptamer was inclined to stay aptamer-target complex rather than the aptamer-cDNA duplex because of robust affinity effects including the electrostatic, hydrogen bond, and spatial matching effects, separating therefore the dsDNA into aptamer-target complex and the cDNA labeled with signal molecules. After that, RecJf exonuclease was added, capable of digesting both cDNA and the aptamer from 5′ termini direction. Attributed to the presence of RecJf exonuclease, liberated was the target from the aptamer-target complex, participating in a new round of association with aptamer in dsDNA duplex and realizing thus the target cycling. Simultaneously, the MB modified on cDNA was released as the same pace of the cDNA being hydrolyzed by exonuclease, getting an easier access to the electrode surface through the host-guest recognition with β-cyclodextrin. Hence strong DPV signal related with the OTA concentration could be detected based on the above principle. Several designing tips were expected to have promoted the performance of this method: (1) it adopted RecJf exonuclease, capable of selectively degrading single-stranded DNA including the aptamer-target complex and its cDNA, thereby engining the target reaction cycles and on-going outputs of signal indicators in sync with the target cycling, due to which a stronger electrochemical readout could be fulfilled. (2) The host-guest recognition with β-cyclodextrin guided the released MB a shortcut to the electrode surface, enriching accordingly the signals detected. (3) As novel type of ssDNA recognition probes, aptamers were employed to ensure the specificity of detection. Hence by this cyclic reaction, a sane electrochemical aptasensor was developed to assay the target.

3.2. Gel electrophoresis

For verification of target recycling induced by the exonuclease, agarose gel electrophoresis was carried out in this strategy. Agarose gel electrophoresis of aptamer (lane 1), cDNA (lane 2), dsDNA (OTA aptamer + cDNA, lane 3), dsDNA (OTA aptamer + cDNA) with RecJf exonuclease (lane 4), aptamer-OTA complex with RecJf exonuclease (lane 5), aptamer with OTA (lane 6), dsDNA digestion product without (lane 7) and with (lane 8) OTA were studied. After 90 min of exonuclease incubation and subsequent 10 min of exonuclease inactivation (incubate at 37 °C), the exonuclease digestion products were acquired. As performed in Fig. 2, two obvious bands were presented respectively in lane 1 and lane 2, which represented the aptamer and the cDNA. Lane 3 displayed the dsDNA alone and lane 4 did dsDNA with RecJf exonuclease whose bands were both evident, manifesting that RecJf exonuclease could not digest dsDNA. But after RecJf exonuclease was added into the OTA aptamer, no band was observed in lanes 5, indicating that the oligonucleotides were degraded into fragments by RecJf exonuclease and then moved out of the gel. Lane 8 revealed a negligible-seen band, suggesting that RecJf exonuclease could make the oligonucleotide of OTA-aptamer digested. Overall, the results demonstrated that the structure of dsDNA could survive itself from being digested by RecJf exonuclease. But the ssDNA such as cDNA and aptamer even in aptamer-OTA complex could be degraded by RecJf exonuclease reflected by agarose gel electrophoresis, implying that the release of OTA involved in reaction cycles was because of the enzymolysis towards OTA-aptamer by RecJf exonuclease.

3.3. Feasibility of the sensing strategy

The feasibility of the RecJf exonuclease-assistant signal amplification for sensitive electrochemical assay of OTA was investigated. Fig. 3 displayed the DPV signal responses prior to and after the OTA addition. Before the participation of the target, the uncleaved aptamer-cDNA was maintained and the hybridization of the aptamer and the MB modified cDNA obstructed the reaction with RecJf exonuclease, thus generating a low DPV background signal (curve a). It was in a great degree because the MB modified on the dsDNA could hardly diffuse freely to the surface of electrode on account of steric hindrance interfered in the electrode

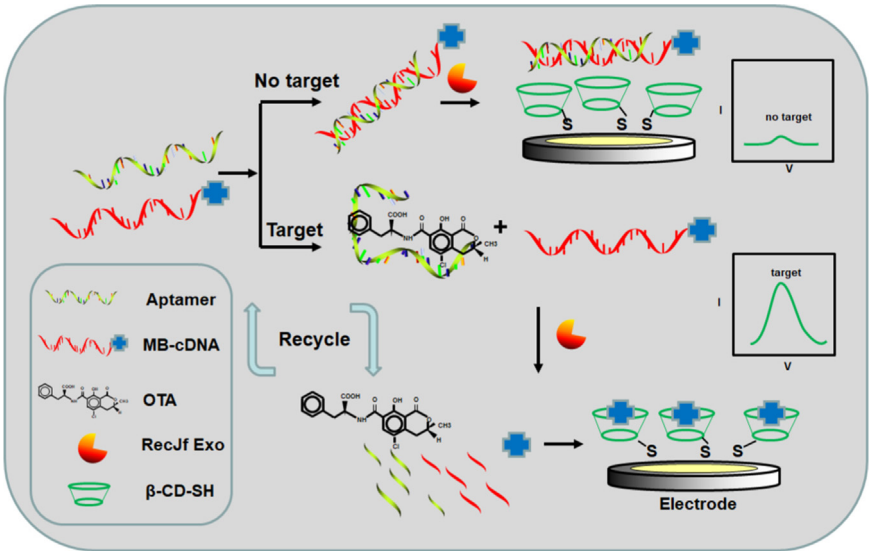

Fig. 1. Sensing principle of aptamer electrochemical platform for OTA detection based on β-cyclodextrin host-guest recognition and exonuclease-assistant signal amplification.

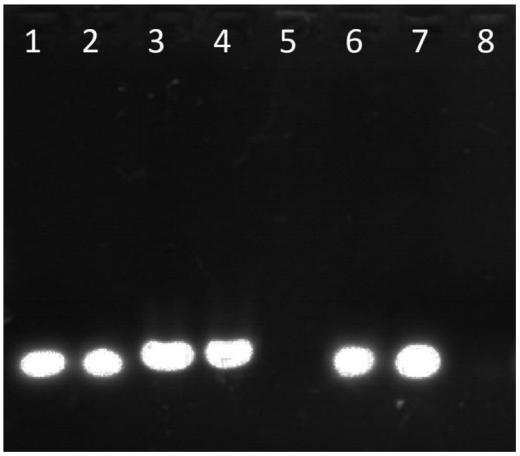

Fig. 2. Gel electrophoresis of aptamer (lane 1), MB-cDNA (lane 2), dsDNA (aptamer + MB-cDNA) (lane 3), dsDNA + RecJf exonuclease digestion product (lane 4), aptamer-OTA + RecJf exonuclease digestion product (lane 5), aptamer with OTA (lane 6), and dsDNA + RecJf exonuclease digestion product without (lane 7) and with (lane 8) OTA.

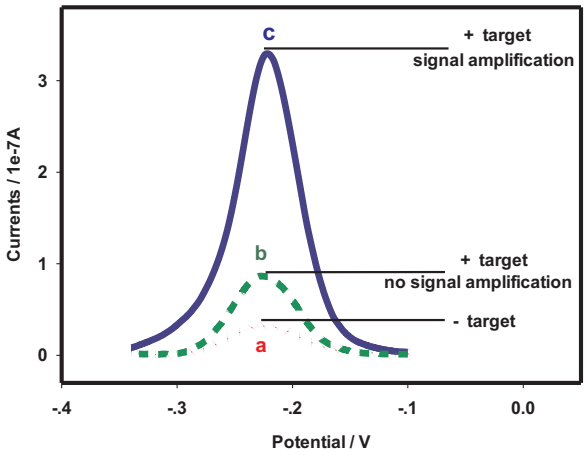

Fig. 3. DPV curves of a) β-CD modified SPE in the absence of OTA, b) β-CD modified SPE after incubation with OTA in the (b) absence and (c) presence of RecJf exonuclease, respectively, and the electrodes were investigated in 0.1 M PBS buffer + 0.1 M KNO_3 (pH 7.4).

and dsDNA. Also, it exhibited a weak enhancement of the DPV readout when OTA existed alone (curve b), principally arisen from the reason that OTA possessed a robust binding affinity towards its aptamer which induced a fraction of dsDNA probes being dissociated, thus causing the release of the MB-cDNA diffusing onto the electrode surface via beta-cyclodextrin host-guest recognition. While in the addition of both the RecJf exonuclease and OTA, the signal responses got steeply increased (curve c) owing to the RecJf exonuclease digestion of the OTA-aptamer complex and cDNA modified with MB, liberating the OTA reused for the next reaction and resulting in a dramatically increasing number of free MB in the solution. Thus feasibility of the proposed strategy was demonstrated substantially.

3.4. Optimization of experimental parameters

Experiment-concerned parameters were optimized respectively such as the concentration of RecJf exonuclease, variables MB-cDNA concentration, digesting time, capturing time between immobilized β-CD and MB for the sake of giving full play to the electrochemical sensing superiority in determination. Firstly, the concentration of RecJf exonuclease was evaluated by the DPV. The oxidation peak current rose with increasing concentration of RecJf exonuclease. But when the RecJf exonuclease concentration was over 2.0 U, not a distinct peak current change was seen, indicating that the maximum concentration was almost reached for cleavage reaction (Fig. 4**A**). Hence the concentration of RecJf exonuclease at 2.0 U was fixed for the following experiments.

To exhibit an optimal electrochemical signal, the MB-cDNA concentration was investigated. The concentrations of the aptamer and MB-cDNA were equal and the electrodes were investigated in 0.1 M PBS + 0.1 M KNO_3 (pH 7.4). The currents of modified electrode after treated with 10 μL mixture containing different MB-cDNA concentration were shown (see Fig. 4**B**), indicating that the current enhanced with an increase of MB-cDNA concentration but began to level off after the MB-cDNA concentration reached to 2 μM. This manifested that the MB-cDNA concentration had approached saturation. Therefore, MB-cDNA concentration of 2 μM was pinpointed as the optimal value.

Since both the RecJf exonuclease and targets were added to the aptasensor for the recycling initiation, so the time of digesting aptamer-target by exonuclease was also tested because it impacted heavily on the sensitivity of this developed system. As displayed in Fig. 4C, the currents intensity increased remarkably along with the enhancement of

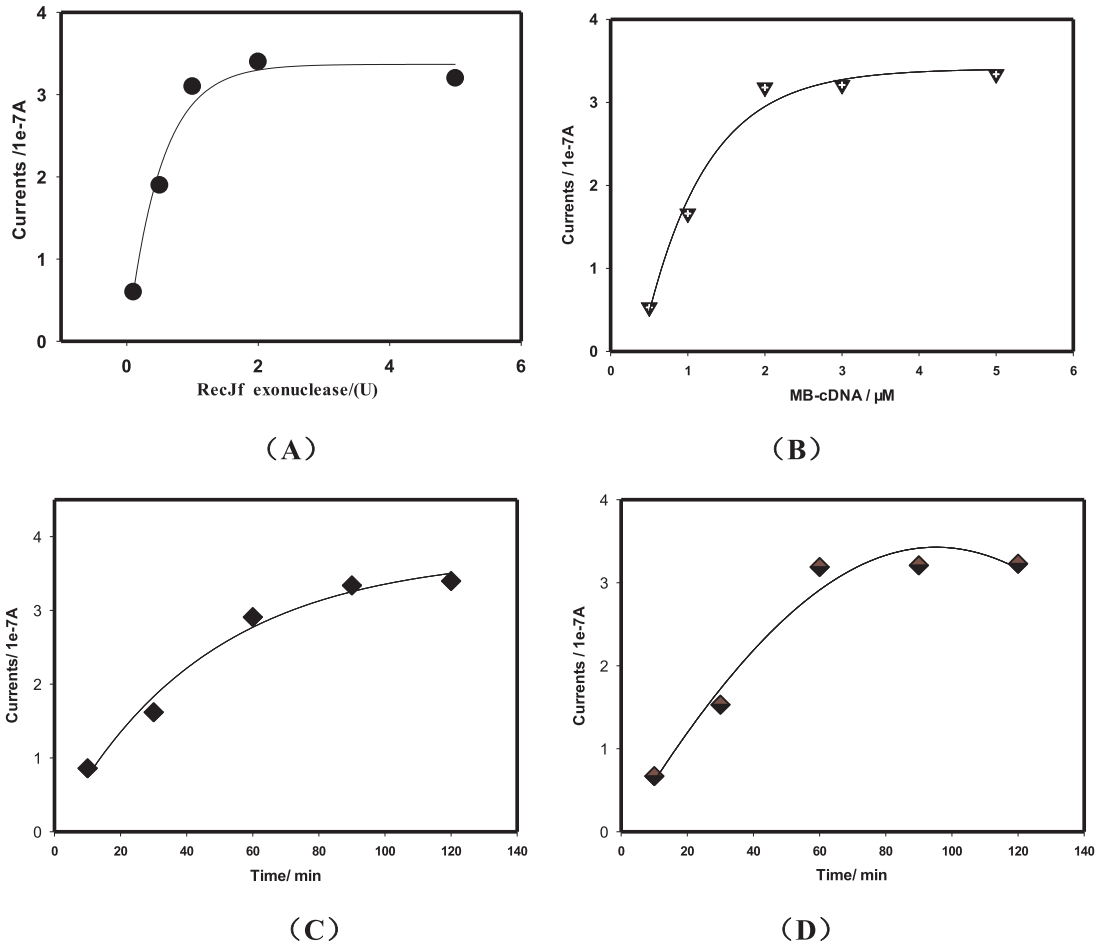

Fig. 4. Influence of the relevant experimental factors on the currents intensity of detection system. (A) RecJf exonuclease concentrations, (B) MB-cDNA concentrations (C) the time of exonuclease digesting and (D) capture time between β-CD and signal molecules. The concentrations of OTA was 10 ng/mL.

incubation time for the aptasensor with target and RecJf exonuclease from 10 min to 2 h. Actually it was turned out that the cleavage process was basically accomplished within 60 min since the continuously increased signal indicated that the RecJf exonuclease catalyzed target recycling process indeed took place and the signal reached saturation at 60 min. But to guarantee the complete cleavage reaction, the time was extended to 90 min. Thus 90 min was regarded as the optimal reaction time for the subsequent experiment.

As another important factor, the capture time between β-CD and MB was also investigated due to the close linkage between the capture efficiency and the detection sensitivity. Hence the capture time was optimized to ensure that the signal molecules were to a maximum extent brought to the electrode surface. As exhibited in Fig. 4D, the current signal increased from 10 min to 120 min and trended to be the top value at 60 min, indicating the capture process was complete. As a result, 60 min was considered to be the optimal capture time.

3.5. Analytical performance of the biosensor

With the optimal preparation of the experimental condition, coupling the compelling host-guest recognition ability of β-cyclodextrin with the preferred exonuclease-assistant signal amplification, the designed electrochemical biosensor was with success fabricated for the sensitive quantification of OTA. Upon the addition of OTA, the MB current was increased and the dynamic range was observed (Fig. 5**A**). The current response increased accordingly with the enhancement of the OTA concentration. Meanwhile, as observed in Fig. 5**B**, the aptamer biosensor's peak current changes kept a linear dependence with the logarithm of the target concentrations ranging from 10 pg/mL to 10.0 ng/mL. The equation was ΔI (1e-7A) = 1.1044 Log C_{OTA} (pg/mL) - 1.0992 (R^2 = 0.9878) and according to the formula S/N = 3, LOD was derived to be 3 pg/mL, where ΔI represented the value that peak current deducted the background signal in the presence of OTA. The satisfying sensitivity was presumably on account of the exonuclease-assistant signal amplification, driving great amounts of MB captured for electrochemical sensing. Furthermore, the other meritorious contributor went to β-cyclodextrin who not only benefited the immobilization of signal probes via host-guest recognition, but also enriched the signals on the electrode surface, thus promoting collectively the sensitive performance of biosensor. Indeed, the proposed method performed better with a lower LOD than others done for OTA determination (Table S1). In terms of these comparisons, the LOD of the approach developed hereby could rival that of novel methods or/and conventional instrument-used methods and immuno-used sensing systems.

3.6. Selectivity test

For evaluation of the specificity of this method, the influence by OTA analogs including AFB1, AFM1, OTB and OTC on the currents intensity was assessed via determining the variations when they interfered in. As shown in Fig. 6, the target could exclusively result in a remarkable current increase tested by the sensing system and there were scarcely any changes in the currents intensity in the presence of other analogs even at as high as 10 times of concentration than that of OTA, manifesting that OTA could be distinguished by proposed sensor

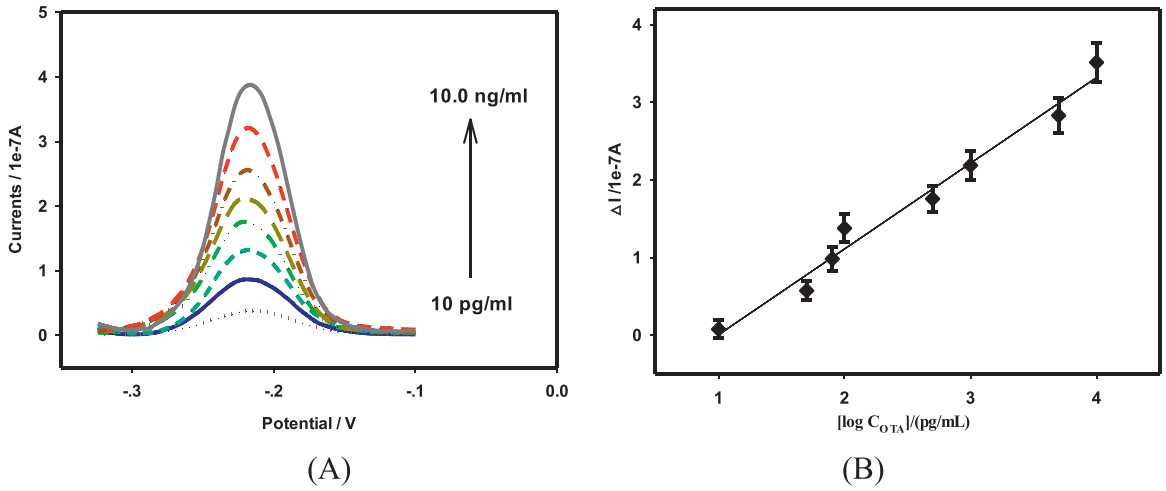

Fig. 5. A) DPV of developed system in the presence of different concentrations of OTA (10, 50, 80, 100, 500, 1000, 5000, 10000 pg/mL) in 100 mM PBS buffer + 0.1 M KNO_3 solution (pH 7.4); B) Linear relationship between currents and target concentrations.

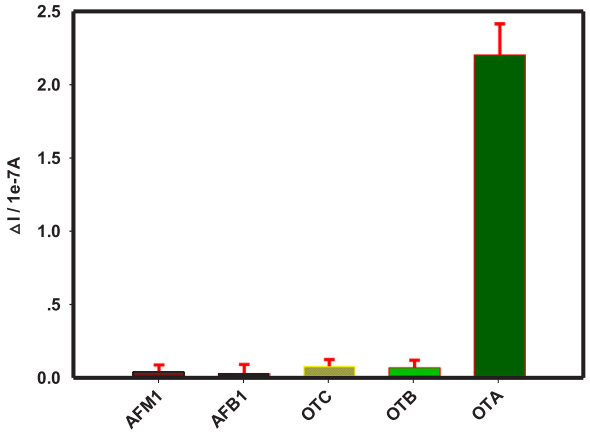

Fig. 6. Specificity of the proposed sensor. a) AFM1, b) AFB1, c) OTC, d) OTB and e) OTA. The concentrations of analogs and OTA were 10 ng/mL and 1.0 ng/mL respectively.

with ease in the presence of OTA analogs, which thus showed a desirable selectivity for target to differentiate analogs.

3.7. Biosensing in actual samples

It was in red wine samples to measure the target for deep validation of the practical application of this constructed analytical platform. The recovery was acquired ranging from 95.6% to 102.7% with RSD (n = 5) between 4.14% and 7.21% after the samples were spiked with target at each level, showing a satisfactory result. In this way, the fabricated electrochemical biosensors might be a competitive candidate for target determination in actual samples with acceptable accuracy.

4. Conclusions

In summary, a sensitive and specific detection for target was achieved by proposed strategy coupled the exonuclease-assisted target recycling via introducing a single target to yield a mass of signals so as to obtaining well performed signal amplification, with utilizing the β-cyclodextrin for the realization of signal molecule enrichment through the host- guest interaction. This assay for OTA showed a wide detection range, low limit, and sound accuracy, which was convenient because it needed neither a complex preparation nor an excessive amount of sample. Compared with the previous-reported methods, this approach was highly sensitive, orientated, easy-operated and significantly efficient, which held a great prospective to be miniaturization and integration for OTA quantification. In a broad sense, this strategy could be further popularized in evaluating extensive ranges of targets due to the flexibility of replacing the corresponding aptamer, thus providing a versatile and universal platform for target detection in real aqueous biological samples with preferable selectivity and sensitivity.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.10.007.

References

Abi, A., Mohammadpour, Z., Zuo, X.L., Safavi, A., 2018. Biosens. Bioelectron. 102, 479–489.
Asadi, M., 2018. Mycotoxin Res. 34, 15–20.
Assaf, H., Azouri, H., Pallardy, M., 2004. Toxicol. Sci. 79, 335–344.
Chen, X., Li, T., Tu, X., Luo, L., 2018. Sens. Actuators B: Chem. 265, 98–103.
Chu, X.F., Dou, X.W., Liang, R.Z., Li, M.H., Kong, W.J., Yang, X.H., Luo, J.Y., Yang, M.H., Zhao, M., 2016. Nanoscale 8, 4127–4133.
Cui, L., Li, Y., Lu, M., Tang, B., Zhang, C., 2018. Biosens. Bioelectron. 99, 1–7.
Dall'Asta, C., Galaverna, G., Bertuzzi, T., Moseriti, A., Pietri, A., Dossena, A., Marchelli, R., 2010. Food Chem. 120, 978–983.
De Santis, B., Debegnach, F., Gregori, E., Russo, S., Marchegiani, F., Moracci, G., Brera, C., 2017. Toxins 9, 169.
Garcia-Fonseca, S., Ballesteros-Gomez, A., Rubio, S., 2016. Anal. Chim. Acta 935, 129–135.
Jiang, C.M., Lan, L.Y., Yao, Y., Zhao, F.N., Ping, J.F., 2018. TrAC Trend Anal. Chem. 102, 236–249.
Jiang, L., Qian, J., Yang, X.W., Yan, Y.T., Liu, Q., Wang, K., Wang, K., 2014. Anal. Chim. Acta 806, 128–135.
Lee, T.P., Saad, B., Salleh, B., Mat, I., 2013. Microchim. Acta 180, 1149–1156.
Lin, C.Y., Zheng, H.X., Sun, M., Guo, Y.J., Luo, F., Guo, L.H., Qiu, B., Lin, Z.Y., Chen, G.N., 2018. Anal. Chim. Acta 1002, 90–96.
Li, Y., Chen, Q., Xu, X.F., Jin, Y.P., Wang, Y., Zhang, L.Y., Yang, W.J., He, L.D., Feng, X.Y., Chen, Y.Q., 2018a. Sens. Actuators B: Chem. 266, 115–123.
Li, J.H., Jiang, J.B., Xu, Z.F., Liu, M.Q., Tang, S.P., Yang, C.M, Qian, D, 2018b. Electrochim. Acta. 260, 526–535.
Li, J.H., Jiang, J.B., Xu, Z.F., Liu, M.Q., Tang, S.P., Yang, C.M., Qian, D., 2018c. Sensor Actuat B: Chem. 260, 529–540.
Ma, C.B., Wu, K.F., Zhao, H., Liu, H.S., Wang, K.M., Xia, K., 2018. Microchim. Acta 185,

347.
Madhurantakam, S., Babu, K.J., Rayappan, J.B.B., Krishnan, U.M., 2018. Biosens. Bioelectron. 116, 67–80.
Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V., 2013. Food Chem. Toxicol. 60, 218–237.
Mayura, K., Parker, R., Berndt, W.O., Phillips, T.D., 1984. Appl. Environ. Microbiol. 48, 1186–1188.
Ni, J., Yang, W., Wang, Q., Luo, F., Guo, L., Qiu, B., Lin, Z., Yang, H., 2018. Biosens. Bioelectron. 105, 182–187.
Pfohl-Leszkowicz, A., Manderville, R.A., 2007. Mol. Nutr. Food Res. 51, 61–99.
Shi, L., Rong, X.J., Wang, Y., Ding, S.M., Tang, W.Y., 2018. Biosens. Bioelectron. 102, 41–48.
Soleas, G.J., Yan, J., Goldberg, D.M., 2001. J. Agric. Food Chem. 49, 2733–2740.
Ventura, M., Vallejos, C., Anaya, I.A., Broto-Puig, F., Agut, M., Comellas, L., 2003. J. Agric. Food Chem. 51, 7564–7567.
Wang, C., Qian, J., Wang, K., Wang, K., Liu, Q., Dong, X., Wang, C., Huang, X., 2015. Biosens. Bioelectron. 68, 783–790.
Wang, Q.L., Chen, M.M., Zhang, H.Q., Wen, W., Zhang, X.H., Wang, S.F., 2016a. Sens. Actuators B: Chem. 222, 264–269.
Wang, Q.L., Chen, M.M., Zhang, H.Q., Wen, W., Zhang, X.H., Wang, S.F., 2016b. Biosens. Bioelectron. 79, 561–567.
Wang, S., Zhang, Y.J., Pang, G.S., Zhang, Y.W., Guo, S.J., 2017. Anal. Chem. 89, 1704–1709.
Wang, Y.J., Nie, J.Y., Yan, Z., Li, Z.X., Cheng, Y., Chang, W.X., 2018. Food Control. 88, 181–189.
Yang, S., You, M., Zhang, F., Wang, Q., He, P., 2018. Sens. Actuators B: Chem. 258, 796–802.
Yu, X.H., Lin, Y.H., Wang, X.S., Xu, L.J., Wang, Z.W., Fu, F.F., 2018. Microchim. Acta 185, 5.
Zhang, H.F., Zhuo, Z.S., Chen, L.J., Chen, C.Q., Luo, F., Chen, Y.T., Guo, L.H., Qiu, B., Lin, Z.Y., Chen, G.N., 2018. Electrochem. Commun. 88, 75–78.
Zhao, Y., Liu, R., Sun, W., Lv, L., Guo, Z., 2018. Sens. Actuators B: Chem 255, 1640–1645.
Zhu, X., Kou, F.X., Xu, H.F., Han, Y.J., Yang, G.D., Huang, X.J., Chen, W., Chi, Y.W., Lin, Z.Y., 2018. Sens. Actuators B: Chem. 270, 263–269.