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Short communication

Wash-free electrochemical aptasensor for the detection of aflatoxins by the signal amplification of ferrocene-capped gold nanoparticles

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This work reported on wash-free renewable electrochemical aptasensor for the rapid detection of aflatoxins using ferrocene (Fc)-capped gold nanoparticle (AuNPs) as the signal reporters. The proximity-dependent electron electron transfer of Fc tags facilitated the achievement of the detection. Specifically, the DNA/Fc-AuNPs captured by the aptamer-modified electrode produced a strong electrochemical peak. In the presence of target aflatoxin, the capture of DNA/Fc-AuNPs by the sensing electrode was prevented due to the formation of aptamer-aflatoxin complexes, thus leading to the decrease in the electrochemical signal. The method exhibits a low detection limit (0.01 pg/mL) for aflatoxin detection with aflatoxin B-1 (AFB1) as a model analyte.

Keywords: aflatoxins; electrochemical aptasensor; gold nanoparticle; ferrocene

1. INTRODUCTION

Aflatoxins are mainly the secondary metabolites produced by Aspergillus flavus and Aspergillus parasiticus. At present, kinds of aflatoxins have been isolated and identified, including aflatoxin B-1 (AFB1), aflatoxin B-2 (AFB2), aflatoxin G-1 (AFG1), aflatoxin G-2 (AFG2), aflatoxin M-1 (AFM1), aflatoxin M-2 (AFM2) and so on [1]. Aflatoxins are highly toxic and carcinogenic. Under humid and hot conditions, aflatoxins are very easy to pollute agricultural products such as corn, peanut, soybean, wheat and nuts [2, 3]. A very small dose of aflatoxins can cause serious harm to people and animals. Taking food with aflatoxins will cause great harm to human, such as dyskinesia, cessation of excretion, hepatitis and jaundice. In serious cases, it can lead to liver cancer, bone cancer, kidney cancer, breast cancer and even death [4]. In view of the great harm of aflatoxins, the standards for the content of

aflatoxins in food and feed has been strictly limited over the world. This makes the detection of aflatoxins become a research hotspot in food and feed [5]. At present, the main methods for the determination of aflatoxins are high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) [6, 7]. Although these methods have high sensitivity and good reproducibility, they are not suitable for rapid and effective detection and analysis of aflatoxins because of their complex procedures, expensive instruments, high detection cost and professional operation.

Electrochemical biosensors have the unique advantages of low cost, wide application range and high degree of automation. They have become one of the most active fields in the analytical community for clinical testing, drug analysis, environmental monitoring, life science and food safety [8-13]. In recent years, a variety of electrochemical biosensors have been constructed with antibodies, enzymes or nucleic acid aptamers as the recognition elements [14-16]. Electrochemical immunosensors and aptasensors are the most widely used methods for the determination of aflatoxins [17]. Moreover, the sensitivity for aflatoxin detection has been greatly improved by the signal amplification of enzymes, rolling ring amplification, DNA self-assembly and nanomaterials [18]. However, these advantages come at the expense of simplicity and rapidity because of a greater number of steps in the electrochemical analysis process. There, it is of great significance to develop a simple, sensitive electrochemical biosensor for the detection of aflatoxins.

Aptamer is a class of single stranded oligonucleotide screened in vitro by systematic evolution of ligand by exponential enrichment (SELEX). It can efficiently and specifically identify and bind with the target, and folds into a special three-dimensional structure, such as stem ring, hairpin, quadrangular ring, pseudoknot and G-quadruplex [19, 20]. Thus, aptamer can distinguish the target from complex mixtures with high specificity. Compared with antibody, aptamer has the advantages of high specificity and stability, good biocompatibility, easy synthesis and modification, long-term preservation and so on. Based on these advantages, aptamers have been widely used in the fields of food, biology, medicine and so on [21, 22]. In this work, we proposed a versatile strategy for the detection of aflatoxins based on the competitive binding between aflatoxin and the detection probe DNA with the aptamer immobilized on the electrode surface. To improve the sensitivity, the signal was amplified by DNA/ferrocene (Fc)-capped gold nanoparticle (AuNPs) (DNA/Fc-AuNPs). As shown in Scheme 1, in the absence of target aflatoxin, the DNA/Fc-AuNPs were captured by the aptamers immobilized on the electrode surface. The bound DNA/Fc-AuNPs could produce a strong electrochemical signal from the oxidation of Fc [23]. In the presence of target aflatoxin, the capture of DNA/Fc-AuNPs by the aptamers would be prevented due to the formation of aptamer-aflatoxin complexes, thus causing the decrease the electrochemical signal. The proximity-dependent electron transfer of Fc tags could distinguish the captured and unbound DNA/Fc-AuNPs without a washing step, thus allowing for the successful development of a wash-free and rapid electrochemical aptasensor for aflatoxin detection.

2. EXPERIMENTAL

2.1 Chemicals and materials

The thiolated AFB_1 aptamer and detection probe DNA were ordered from Sangon Biotech. Co., Ltd. (Shanghai, China). The sequences of aptamer, detection probe DNA and random DNA are 5'-HS-

$(CH_2)_6$-GCACGTGTTGTCTCTCTGTGTCTCGTGC-3', 5'-HS-$(CH_2)_6$-GCACGAGACACAGAG-3' and 5'-HS$(CH_2)_6$-TCAACATCAGTCTGATAAGCTA-3', respectively. The water-soluble Fc-labeled Asp-Cys peptide (Fc-DC) was provided by ChinaPeptides Company (Shanghai, China). AFB1, AFM1, ochratoxin A (OTA) and tris(2-carboxyethyl)phosphine (TCEP) were obtained from Sigma-Aldrich (Shanghai, China). AFB2, AFG1 and zearalenone (ZON) were provided by Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). AuNPs were ordered from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). The Tsingtao beer was purchased from the local market. All the reagents were used without additional purification.

2.2 Fabrication of DNA/Fc-AuNPs

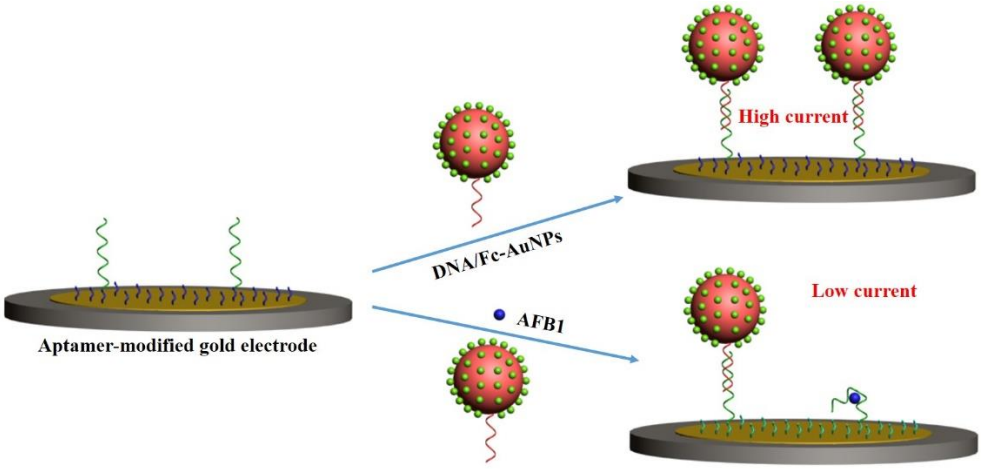
The DNA probe and Fc-DC were immobilized on the surface of AuNPs through the Au-S interactions [24]. Briefly, 450 μL of AuNPs were mixed with 25 μL of phosphate buffer (10 mM, pH 7.2) containing 200 nM DNA detection probe and 500 μM TCEP. After reaction for 1 h, 25 μL of 50 μM Fc-DC in the buffer was added to the mixed solution. The modified AuNPs were collected by centrifugation and then washed three time. To remove any traces of unreacted detection probe DNA, the resulting DNA/Fc-AuNPs were incubated with a gold film for 24 h, and then diluted to 1 mL with Tris buffer (10 mM, pH 7.4). The modified DNA/Fc-AuNPs were characterized by Cary 60 spectrophotometer, transmission electron microscopy (TEM) and dynamic light scattering (DLS).

2.3 Preparation of aptamer-modified electrodes

The gold electrodes were polished with 50 nm alumina powder. After being rinsed with 50% ethanol, the electrodes were placed in 0.5 M H_2SO_4 for consecutive cyclic voltammetric scanning. The potential changed in the range of −0.2 ~ 1.3 V with a scan rate of 0.1 V/s. When a negligible current change was observed, the electrodes were washed with phosphate buffer and then incubated with the mixture of 1 μM aptamer-AFB_1 complex and 500 μM TCEP. After incubation for 6 h, the electrodes were rinsed thoroughly with 10 mM HCl and water and then immersed in 1 mM cysteine solution for 30 min. This step can release the bound AFB_1 and block the unreacted gold surface. Moreover, cysteine exhibiting good antifouling property and biocompatibility can eliminate the nonspecific adsorption. The aptamer-modified electrodes were kept at 4 ^oC for use.

2.4 Electrochemical detection of AFB1

The aptamer-modified electrode was firstly incubated with 25 μL of different concentrations of AFB1 in 50 mM Tris buffer (pH 7.4) containing 1 mM EDTA and 20 mM NaCl. Then, 25 μL of the as-prepared DNA/Fc-AuNPs were added to the sample solution. After incubation for 1 h at room temperature, differential pulse voltammetry (DPV) measurement was carried out on a CHI 660E electrochemical workstation (CH Instruments, Shanghai, China). The auxiliary and reference electrodes are platinum wire and Ag/AgCl electrode, respectively. For the real sample assays, the beer was diluted 50 times by Tris buffer. Then, AFB1 with a known concentration was added to real sample. The other procedures were the same as those for the assays of AFB1 standard samples.



Scheme 1. Principle of the electrochemical aptasensor for AFB1 detection.

3. RESULTS AND DISCUSSION

3.1 Characterization of DNA/Fc-AuNPs

AuNPs are the commonly used carriers for the immobilization of detection probes and signal reporters because of their advantages of easy modification, uniform size and shape, long-term stability, excellent conductivity and good catalytic property [25]. Thus, AuNPs were employed to load the DNA detection probes and electroactive Fc-DC tags. The resulting DNA/Fc-AuNPs were characterized by UV-vis spectrophotometer and TEM (Figure 1A). The DNA/Fc-AuNPs exhibited a typical maximum absorption at 545 nm, which is ascribed to the surface plasmon resonance of dispersed AuNPs. The TEM result suggests that the DNA/Fc-AuNPs were monodispersed (the inset). The average diameter of AuNPs increased from 84 to 101 nm after the modification of DNA and Fc-DC (Figure 1B). The zeta potential changed from −22.5 to −18.7 mV. These results are indicative of the successful preparation of DNA/Fc-AuNPs.

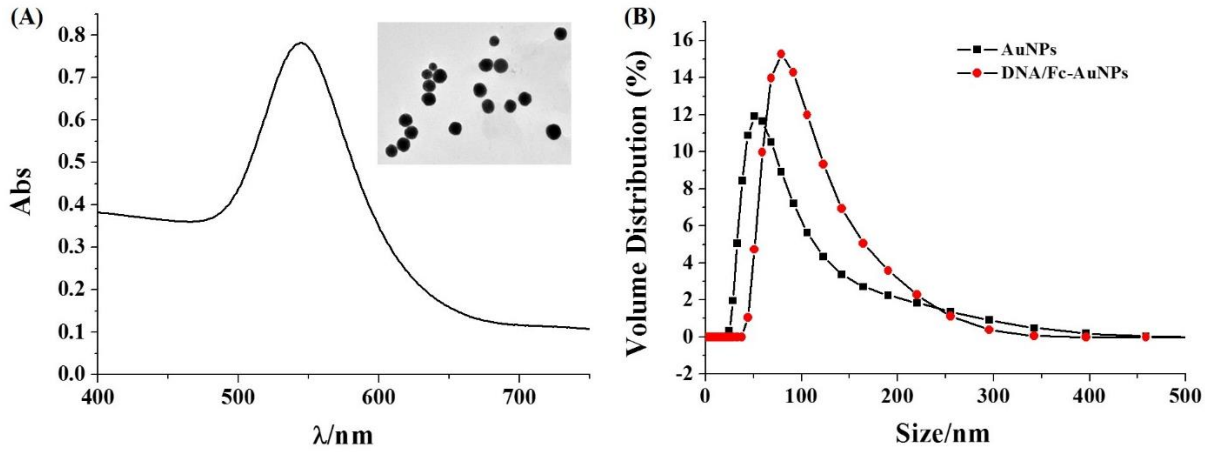


Figure 1. (A) UV-vis spectra and TEM image of DNA/Fc-AuNPs. (B) Size distribution of AuNPs and DNA/Fc-AuNPs

3.2 Feasibility

To prove the feasibility of the aptasensor, the aptamer-modified electrodes were treated by different solutions and then the DPV curves were collected. As depicted in Figure 2, no redox peak was observed at the aptamer-modified electrode (curve a). However, when the sensor electrode was incubated with DNA/Fc-AuNPs in the absence of AFB1 (curve b), a remarkable oxidation peak was observed. There was no redox peak at the aptamer-modified electrode (curve c) when it was incubated with Fc-AuNPs. A control experiment was performed by incubation of the random DNA-modified electrode with DNA/Fc-AuNPs (curve d). As a result, no redox peak was observed. These results indicate that the signal production was dependent upon the capture of DNA/Fc-AuNPs through the hybridization between the aptamer on electrode surface and the detection probe on AuNPs. Thus, the wash-free aptasensor can be developed by the proximity-dependent electron transfer of Fc tags. We also found that the relative standard deviation (RSD) obtained at three electrodes was about 4%, suggesting that multiple electrodes could be used for the detection of different samples.

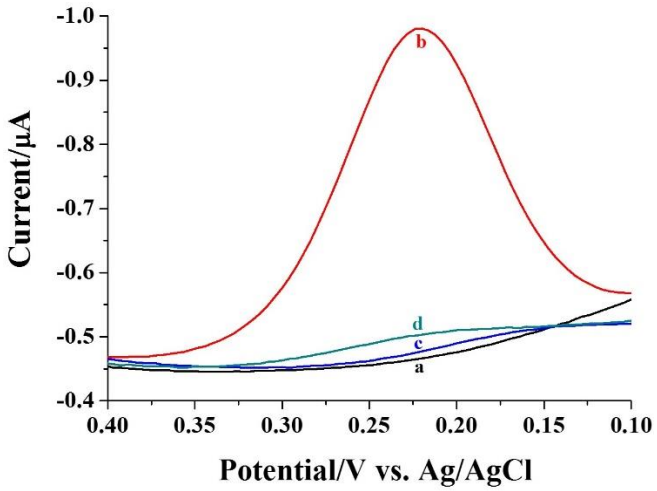


Figure 2. DPV curves of aptamer-modified electrode in the absence (curve a) and presence of DNA/Fc-AuNPs (curve b) or Fc-AuNPs (curve c). Curve d corresponds to the DPV curve of the random DNA-modified electrode in the presence of DNA/Fc-AuNPs.

3.3 Sensitivity

To evaluate the sensitivity of the aptasensor, the aptamer-modified electrodes were incubated with DNA/Fc-AuNPs in the presence of different concentrations of AFB1. As shown in Figure 3A, the oxidation peak current (I_{pa}) decreased gradually with the increase of AFB1 concentration. Figure 3B depicts the dependence of I_{pa} on AFB1 concentration. The RSDs for the detection of various samples were all lower than 9%, which is indicative of good reproducibility of the aptasensor. In the range of 0.01 ~ 7.5 pg/mL, the curve showed a good linear relationship with an equation of I_{pa} = 0.47 – 0.057[AFB1] (pg/mL). The detection limit of 0.001 pg/mL is greatly lower than that of the safe threshold. The value is comparable to that achieved by other electrochemical biosensors (Table 1) [26-

34]. Such high sensitivity can be attributed to the signal amplification of AuNPs and the well-defined oxidation peak of Fc. Moreover, the aptasensor is wash-free, thereby shortening the analysis time and decreasing the detection complexity.

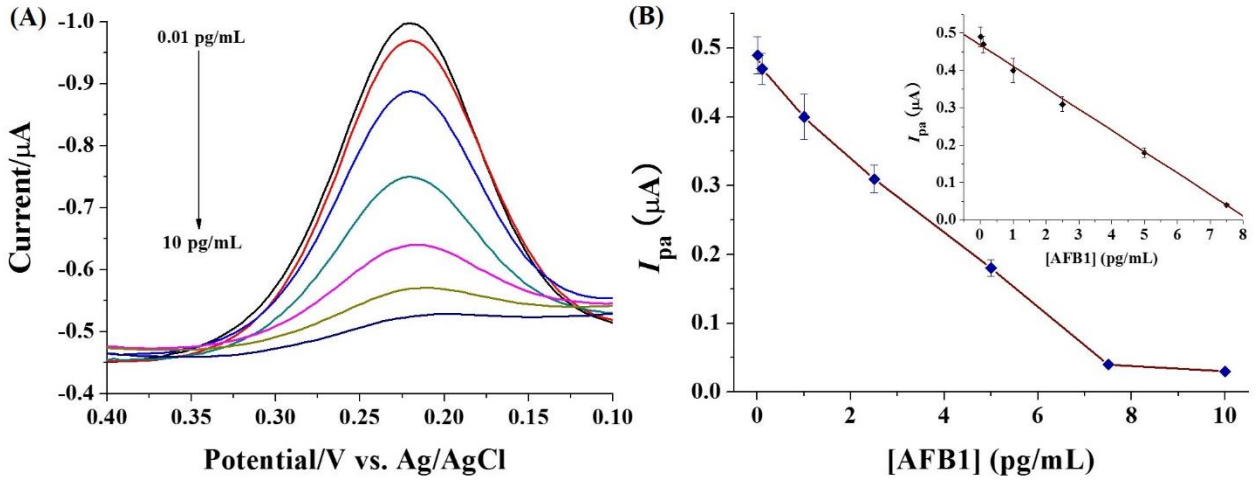


Figure 3. (A) DPV curves for the detection of various concentrations of AFB1 (from top to bottom: 0.001, 0.01, 0.1, 0.25, 0.5, 0.75 and 1 pg/mL). (B) Dependence of I_{pa} on AFB1 concentration. The inset shows the linear part of the curve.

Table 1. Analytical performance of different aptasensors for AFB1 detection.

Method	Signal labels	Detection limit	Linear range	Reference
ACV	Fc-aptamer	12 fg/mL	0.1 pg/mL – 10 ng/mL	[26]
ACV	Fc-aptamer	0.01 pg/mL	10 fg/mL – 50 ng/mL	[27]
DPV	MB-aptamer	6 pM	1 – 625 nM	[28]
DPV	CuNPs	6.75 aM	0.1 fM – 100 pM	[29]
PEC	AgInS2-QDs	0.608 fg/mL	1 fg/mL – 1 ng/mL	[30]
PEC	AuNPs	0.01 ng/mL	30 pg/mL – 200 ng/mL	[31]
ECL	HRP/Au NRs	0.12 pM	5 pM – 10 nM	[35]
ECL	EPDNs	0.27 pg/mL	1 pg/mL – 5 ng/mL	[32]
EIS	SA-biotin-FNPs	0.05 pg/mL	0.05 ~ 3 pg/mL	[33]
DPV	Fc-AuNPs	0.01 pg/mL	0.01 ~ 7.5 pg/mL	This work

Abbreviation: ACV, alternating current voltammetry; PEC, photoelectrochemistry; ECL, electrochemiluminescence; EIS, electrochemical impendence spectra; MB, methylene blue; CuNPs, copper nanoparticles; QDs, quantum dots; HRP/Au NRs, horseradish peroxidase-modified gold nanorods; EPDNs, enzyme-driven programmable assembled 3D DNA nanoflowers; SA, streptavidin; biotin-FNPs; biotinylated biotin-phenylalanine nanoparticles.

3.4 Selectivity

To test the selectivity of the aptasensor, other aflatoxins including AFB2, AFG1, AFM1, OTA and ZON were determined. As shown in Figure 4, no significant decrease in I_{pa} was observed even if the concentrations of other aflatoxins were 20 times higher than that of AFB1. To investigate the anti-

interference, the mixture of AFB1 and other aflatoxins was determined. There is no significant difference in I_{pa}, demonstrating that other aflatoxins has little effect on AFB1 detection. Because the aptasensor is highly sensitive, the selectivity could be further improved by diluting the real samples if other components can cause an interference for the target detection. Moreover, we found that the sensing electrode could be readily regenerated by removing the captured aptamer or DNA/Fc-AuNPs by 10 mM HCl. No significant decrease in the detection ability was found after seven regeneration cycles. Thus, one electrode could be used for the assays of multiple samples, reducing the detection cost.

In order to evaluate the applicability of the sensor, beer samples spiked with three concentrations of AFB1 were analyzed. The found values were closed to those of the spiked AFB1 (Table 2). The recoveries ranged from 97 to 110%, indicating that the method is capable to detect AFB1 in real sample.

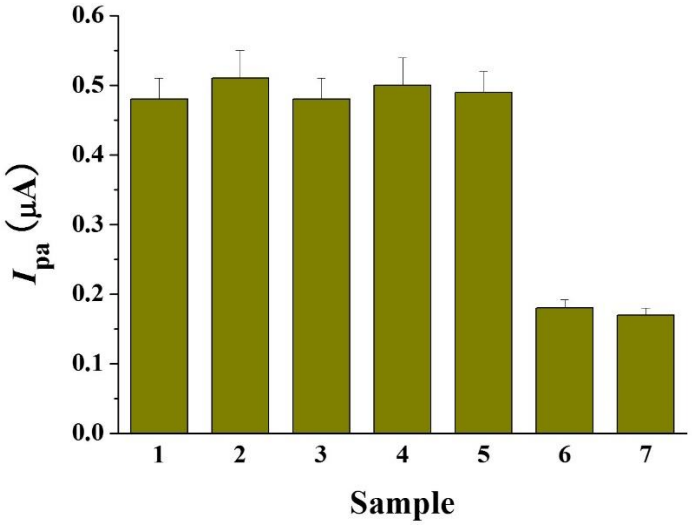


Figure 4. Selectivity towards AFB2 (bar 1), AFG1 (bar 2), AFM1 (bar 3), OTA (bar 4), ZON (bar 5), AFB1 (bar 6) and the mixture of 1 ~ 6 (bar 7). The concentration of AFB1 was 5 pg/mL and that of others is 100 pg/mL.

Table 2. Results for the detection of AFB1 in beer.

Spiked AFB1 (pg/mL)	Found AFB1 (pg/mL)	Recovery (%)	RSD (%)
0.10	0.11	110	9.6
1.00	0.97	97	7.5
5.00	4.93	98.6	7.2

4. CONCLUSION

In this work, we developed a wash-free, renewable electrochemical method to detect aflatoxins with the signal amplification of Fc-capped AuNPs. Based on the proximity-dependent electron transfer of redox reporters, the bound and unbound DNA/Fc-AuNPs have been distinguished without cleaning

steps. The method can detect AFB1 as a model analyte with high sensitivity and selectivity. We believe that the strategy can be used to detect other aflatoxins by using a sequence-specific aptamer.

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