Sensors & Actuators: B. Chemical 276 (2018) 1–7

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Ultrasensitive aptasensor with DNA tetrahedral nanostructure for Ochratoxin A detection based on hemin/G-quadruplex catalyzed polyaniline deposition

Min Wei[a,b,*], Wenyang Zhang[a]

[a] College of Food Science and Technology, Henan University of Technology, Zhengzhou, 450001, PR China
[b] Henan Key Laboratory of Cereal and Oil Food Safety Inspection and Control, Henan University of Technology, Zhengzhou, 450001, PR China

ARTICLE INFO

Keywords:
Aptasensor
DNA tetrahedral nanostructure
Hemin/G-quadruplex
Polyaniline deposition
Ochratoxin A

ABSTRACT

An ultrasensitive electrochemical aptasensor with DNA tetrahedral nanostructure (DTN) for Ochratoxin A (OTA) detection was developed based on hemin/G-quadruplex catalyzed polyaniline (PANI) deposition in the presence of H_2O_2. DTN, which modified with OTA aptamer on top vertex and thiol group on other three vertices, was constructed on the modified electrode surface via Au-S bond and prominent template for PANI deposition owing to their numerous negative charges. The prepared gold nanorods (AuNRs) was used as a carrier to immobilize DTN and promote electron transfer. In the presence of OTA, the aptamer specific for OTA was formed G-quadruplex structure. The hemin/G-quadruplex was formed when hemin was intercalated into G-quadruplex, which had high catalytic activity to catalyze PANI deposition in the presence of H_2O_2 and amplify DPV signal. The amount of generated PANI was dependent on the number of hemin/G-quadruplex induced by OTA. Therefore, DPV currents was related to the concentration of OTA. The fabricated aptasensor exhibited a good linear response toward OTA over a wide range of concentrations from 0.001 ng/mL to 0.5 ng/mL, and the limit of detection of the proposed aptasensor was 0.26 pg/mL.

1. Introduction

Food contaminants by mycotoxin have become an increasing food safety and public health concern all over the world. As one of the key mycotoxin, Ochratoxin A (OTA), secreted by Aspergillus and Penicillium, has attracted more attention due to its nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic activities [1,2]. OTA is found as a contaminant widespread in food products, including maize, rice, wheat, peanut, coffee, bean and dried food. For these reasons, OTA was classifical as a class 2B carcinogen in 1993 by the International Agency for Research on Cancer, and many countries have set a maximum permitted OTA content in food. Some directives have been introduced by the ministry of the Health of People's Republic of China in order to set the maximum limit for OTA in foodstuffs such as cereal (5.0 μg/kg), cereal-derived products (5.0 μg/kg), wine (2.0 μg/kg), and dried fruits (5.0 μg/kg) (Ministry of Health 2017). Therefore, it is importantto develop a rapid, precise, selective, low-cost and on-site method for the detection of OTA in the control of food safety and quality. In practice, high performance liquid chromatography, gas chromatography, high performance liquid chromatography tandem mass spectrometry are popular by the reason of their good accuracy, high sensitivity, and excellent reproducibility [3–6]. However, these analytical methods exist some drawbacks including tedious procedures, expensive and sophisticated equipment, so, they are unsuited for the on-site assay. In recent years, electrochemical assay based on biosensor has been widely studied for OTA detection because its equipment is inexpensive and simple, and easy to realize miniaturization.

Aptamer, an artificial single strand nucleic acid ligand, has merits including high affinity and specificity, excellent stability, easy to synthesize and modify [7,8]. In the presence of OTA, the aptamer specific for OTA was switched from a random coil to an antiparallel G-quadruplex structure. Recently, G-quadruplex has been intensively applied in aptasensor due to that formation of the G-quadruplex structure can cause signal change under certain conditions [9]. However, the existed main problem is the grasp of density and orientation of biomolecules on the electrode surface. As a novel recognition probe, DNA tetrahedral nanostructure (DTN) has attracted an increasing number of interests. DTN could be immobilized on gold electrode surface by modifying three of its vertices with thiol groups, resulting in a 5000-fold greater affinity than single point-tethered oligonucleotides. The fourth vertex

* Corresponding author.
E-mail address: wei_min80@163.com (M. Wei).

https://doi.org/10.1016/j.snb.2018.08.072
Received 15 March 2018; Received in revised form 1 August 2018; Accepted 14 August 2018
Available online 16 August 2018
0925-4005/ © 2018 Published by Elsevier B.V.

was designed with an extended DNA anchor probe as capture which was complementary to partial of the target [10–12]. This DTN structure can control the assembled probe DNA on the electrode interface and rise above the spatial eff ;ect of a conventional single-stranded probe to increase accessibility and reactivity. Taking the advantages of well-controlled density and high mechanical rigidity, the application of DTN not only effectively eliminates the non-specific adsorption, but also reduces the inter-strand interaction. [13,14]. Zuo's group reported DTN-based electrochemical biosensor for MicroRNA detection with encouraging outcomes [15]. What's more, when hemin/G-quadruplex was grafted to DTN, the scaffold enhanced the catalytic activity of DNAzyme [16]. Wei's group reported a label-free ultrasensitive assay of 8-hydroxy-2′-deoxyguanosines via DTN, which improved the sensitivity to 10-fold compared to their previously reported method without DTN [17].

Polyaniline (PANI) becomes more and more popular electrochemical probe that be used in the fields of electrocatalysis and electrochemical sensors, due to its remarkable conductivity and redox property, good environmental stability as well as mildly polymerized conditions [18,19]. Nucleic acid backbones of DNA with plentiful negative charges are excellent templates for PANI deposition. Horseradish peroxidase (HRP) mimicking DNAzymes, which consist of hemin/G-quadruplex complexes, can efficiently catalyze oxidation polymerization of aniline to PANI in the presence of H_2O_2 [20,21]. Compared with HRP, the HRP mimicking DNAzymes have the advantages of easy synthesis, high stability, low cost. It is widely applied to construct electrochemical, fluorescent, colorimetric sensing platform [22–25].

In recent years, gold nanorods (AuNRs) have been intensively used in different biosensors for signal amplification due to their large specific surface area, high catalytic properties as well as good conductivity and biocompatibility [26–29].

In this work, according to the formation of G-quadruplex structure along with the combination of OTA and its specific aptamer, an ultrasensitive electrochemical biosensor platform based on hemin/G-quadruplex catalyzed PANI deposition in the presence of H_2O_2 for signal amplification was developed. DTN with OTA aptamer at one vertex and modified with thiol group on other three vertices was constructed on the modified electrode surface to rise above the spatial eff ;ect of a conventional single-stranded probe and increase accessibility and reactivity. The prepared AuNRs was used to modify aminated Au electrode (NH_2-AuE), which not only enhmanced the loading of DTN but also effectively improved the electron transfer rate. In the presence of OTA, the aptamer specific for OTA was formed G-quadruplex structure. The hemin/G-quadruplex were formed when hemin was intercalated into G-quadruplex, which had high catalytic activity to catalyze PANI deposition in the presence of H_2O_2 and amplified current signal. The amount of generated PANI was dependent on the number of hemin/G-quadruplex that induced by OTA. Therefore, the current signals were related to the concentration of OTA.

2. Experimental

2.1. Materials and reagents

All oligonucleotides were synthesized by Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). The sequences were shown in Table 1. OTA was purchased from Sigma-Aldrich Chemical Co.Ltd. (St. Louis, MO). All other chemicals were purchased from Glycitein (Shanghai, China). All solutions were prepared with deionized water (Milli-Q, Millipore, 18.2 MΩ).

2.2. Apparatus and electrochemical measurements

All the electrochemical experiments were performed on a CHI 660E Electrochemical Workstation (Shanghai Chenhua Instrument Corporation, China). A three-electrode system was employed with Au electrode (AuE, the diameter of 3 mm) as the working electrode, platinum wire as the auxiliary electrode, and Ag/AgCl as the reference electrode. The electrochemical signal was measured with CV by scanning from −0.3 V to 0.3 V and DPV by scanning from −0.2 V to 0.2 V. EIS was recorded with a frequency range of 0.01 to 10^6 Hz.

Scanning electron microscopy (SEM) was performed using a JEOL JSM7100 F SEM facility (Jeol, Japan). Transmission electron microscopy (TEM) was performed using a JEOL JEM-100SX TEM (JEOL, Japan).

2.3. Preparation of AuNRs

The AuNRs was prepared according to the previous report [30,31]. The seed solution was prepared by keep the solution containing 250 μL of 10 mM $HAuCl_4$, 9.75 mL of 100 mM CTAB, 600 μL of 0.01 M $NaBH_4$ at 25 ℃ for 40 min. The growth solution containing 49 mL of water, 0.912 g of CTAB, 0.11 g of 5-bromosalicylic acid, 240 μL of 20 mM $AgNO_3$, 500 μL of 50 mM $HAuCl_4$ solution, 130 μL of 100 mM ascorbic acid was made. Finally, 80 μL of seed solution was injected into the growth solution. The resultant mixture was stirred for 30 s and left undisturbed at 30 °C for 12 h for AuNRs growth.

2.4. Fabrication hemin/G-quadruplex/DTN/AuNRs/NH_2-AuE sensor

The AuE (diameter of 3 mm) was soaked in Piranha solution (H_2SO_4/H_2O_2 = 7/3) for 15 min and polished with 0.05 μM γ Al_2O_3. Then, the AuE was rinsed with ultrapure water. In order to make AuNRs better fixed on the surface of electrode, the electrode surface was derived. The electrode surface derivatization was performed according to the literature report [32]. The AuE was scanned by CV in 1 mM 4-nitrobenzenediazonium tetrafluoroborate containing 100 mM tetrabutylammonium tetrafluoroborate. After the formation of p-nitrophenyl modified AuE, it was scanned negative to reduce the nitro group to amine in 10 mM HCl for preparation of p-aminophenyl modified AuE (NH_2-AuE). Then, 4 μL AuNRs was dropped on the surface of NH_2-AuE, and the AuNRs/NH_2-AuE was obtained after dried at room temperature.

The DTN was formed according to the reported literature [17]. In short, equimolar quantities of four strands (Table 1) were dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) to a final concentration of 50 μM, respectively. Four strands (1 μL of each strand) were mixed with 1 μL of TCEP (500 mM) and 45 μL of TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0). The mixed solution was heated 95 °C for 8 min and then cooled to 4 °C for more than 30 min. 3 μL of DTN was dropped on the modified electrode surface for 12 h at 37 °C in the constant temperature and humidity chamber. By this means, DTN with OTA aptamer at one vertex and modified with thiol group on other three vertices was constructed on the modified electrode surface via Au-S bonds. Next, various concentration of OTA were added to the modified electrode surface for 12 min at 37 °C in the constant temperature and humidity chamber. Then the above modified electrode were incubated in TK buffer (20 mM Tris, 50 mM KCl, 2.5 μM hemin, pH 8.0) for 40 min at room temperature. Followed by washing with Tris−HCl (pH 7.4) to remove the unbound OTA, the hemin/G-quadruplex with peroxidase catalytic activity were formed. For the deposition of PANI, the resulting modified electrode was incubated in deposition buffer (100 mM HAc-NaAc, pH 4.3, 100 mM H_2O_2, 10 mM KCl, 100 mM aniline) for 90 min at room temperature.

2.5. Reproducibility and repeatability of the electrochemical aptasensor

To evaluate the reproducibility of the proposed biosensor, five fabricated aptasensors were tested by monitoring the current signal with 1 ng/mL OTA under the same experimental conditions. To evaluate the repeatability of the proposed biosensor, the aptasensor was investigated by monitoring the current signal in the presence of OTA (1 ng/mL) for five replicate determinations under the same condition.

Download English Version:

https://daneshyari.com/en/article/8947623

Download Persian Version:

https://daneshyari.com/article/8947623

Daneshyari.com