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Label-free ochratoxin A electrochemical aptasensor based on target-induced noncovalent assembly of peroxidase-like graphitic carbon nitride nanosheet

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ABSTRACT

The development of simple, fast and sensitive aptasensor for OTA plays an important role in food safety due to its severe toxicity. In this research, taking advantage of the intrinsic peroxidase-like activity of g-C_3N_4 nanosheet (g-CNNS) and its high affinity toward signal-strand DNA, a label-free electrochemical OTA aptasensor was fabricated. There is an outstanding property that the aptasensor did not require labeled aptamer and immobilization of g-CNNS compared with previous g-CNNS-based aptasensors. The presence of OTA induced the conformational change of the aptamer, and then g-CNNS noncovalently assembled with the free complementary strand on the electrode via π-π interaction. The captured g-CNNS catalyzed the oxidation of H_2O_2 to amplify current signal. As a result, this aptasensor realized the detection limit of 0.073 nM, and was also employed to assay OTA in the real samples, including red wines, juices and corns. More importantly, the development of this label-free electrochemical aptasensor opens up the application of g-CNNS as the peroxidase-mimicking DNAzyme.

1. Introduction

Recently, two-dimensional (2D) graphite-like carbon nitride nanosheet (g-CNNS), a metal-free semiconductor nanomaterial [1,2], has attracted considerable interest due to its unique electronic and optical properties. This layered material has been employed to create biosensors, including photoelectrochemistry [3–6], electrochemiluminescence [7–14], and fluorescence [15–20]. These biosensors have been used to recognize various targets, such as metal ions [8,17], DNA/RNA [10], small molecules [19], and proteins [7,13,16], even cell image [15,20]. Besides electronic and optical properties, g-CNNS also exhibits peroxidase-like activity, which is only applied as the mimetic enzyme to assay H_2O_2 and glucose [21,22]. In above sensors, there are two main methods to obtain the detectable signal. The first one, g-CNNS is used as the platform to provide electrochemical or electroluminescent signal, and then combined with other signal markers including fluorophores, enzymes, electroactive species, and other nanomaterials to induce the signal change. However, these markers were usually labeled with the recognition elements (aptamers or antibodies), which might affect the hybridization and recognition of the recognition elements towards target. The second one, functionalized g-CNNS was immobilized on the electrode, followed by the modification of recognition elements. Unfortunately, the immobilized g-CNNS were liable to be polluted and exfoliate during the following modifications, which influenced the experimental results. Therefore, there is a question raised whether the label-free biosensor can be fabricated without immobilization of g-CNNS.

Ochratoxins, secondary metabolites secreted by fungi species (e.g. Aspergillus and Penicillium) during their growth, are present in various crops and beverages including coffee, wine, grape juice and dried fruits [23,24]. Among them, ochratoxin A (OTA) is classified as a possible carcinogen by the International Agency for Research on Cancer (IARC) due to its severe toxicity. Worse, OTA is chemically stable, so that it is metabolized very slowly with a half-life of more than 30 days in the

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body. With the recognition of its serious threat, the development of sensing platform for OTA plays a vital role in food safety. The researchers have made great efforts for the monitor of OTA, such as high performance liquid chromatography (HPLC) [25–29] and enzyme-linked immunosorbent assays (ELISA).

Aptamers are single-strand nucleic acids selected by systematic evolution of ligands by exponential enrichment (SELEX). Compared with antibody, they possess the following advantages: simple synthesis, easy labeling, and good stability, which have attracted the interest of researchers. Since the aptamer for OTA was selected in 2008 [30], various ingenious aptamer-based sensors (aptasensors) have been fabricated for OTA, including fluorescence [31–35], electrochemical [36–42], colorimetric [43–46], and piezoelectric QCM [47]. Among them, electrochemical technology is an effective approach due to its low cost, simple devices, and wonderful compatibility with advanced micromachining technologies. In order to improve the sensitivity of electrochemical aptasensors, various nanomaterials with unique features and functions have been introduced, including semiconductor quantum dots (QDs) [42], carbon nanomaterials [37,40], and noble metal nanomaterials [48].

It is well-known that g-CNNS have high affinity towards ssDNA rather than dsDNA [18]. Based on this phenomenon, various fluorescence [18] and ECL [49] aptasensors have been developed. Inspired by these, for the first time, a simple and label-free aptasensor based on target-induced noncovalent assembly of g-CNNS was fabricated for OTA. In this aptasensor, the presence of OTA induced the conformational change of aptamer to release free ssDNA. And then the introduced g-CNNS noncovalently assembled with free ssDNA via π-π interaction, and as mimetic enzyme catalyzed the oxidation of H_2O_2 to generate current. By this aptasensor, the detection limit of 0.073 nM was achieved. Furthermore, this aptasensor exhibited excellent performance towards the analytical application in drinks, including red wines and juices. This electrochemical aptasensor not only realizes the monitor of OTA, but also opens up the application of g-CNNS as the peroxidase mimics.

2. Experimental sections

2.1. Materials and reagents

All the synthetic oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China). The sequences of oligonucleotides were as follows:

Aptamer: 5′-GATCGGGTGTGGGTGGCGTAAAGGGAG CATCGGACA-3'

Complementary DNA (cDNA): 5-SH-TGT CCG ATG CTC CCT TTACGC CAC CCA -3‘

Dicyanamide, OTA (10 μg/mL in acetonitrile, MW 408.84), ochratoxin B (OTB, 10 μg/mL in acetonitrile, MW 369.37), 6-mercaptohexanol (MCH), and Tris(2-carboxyethyl) phosphinehydrochloride (TCEP), were purchased from Sigma. All reagents were of analytical reagent grade and used without further purification. All aqueous solutions were prepared with doubly distilled water, which has been purified with the Milli-Q purification system.

2.2. Synthesis of g-CNNS

The CNNS were prepared according to the previously literatures [17,50]. Firstly, 3 g of dicyanamide was placed in a tube furnace (GSL 1400X, Kejing Materials Technology Lt. Co., Hefei, China) and heated at 600 °C for 2 h under air condition with a ramp of about 3 °C/min for both the heating and cooling processes. After these, the pale yellow bulk g-C_3N_4 was obtained. Subsequently, 100 mg of bulk g-C_3N_4 powder was ground well with a mortar and a pestle, and dispersed in 100 mL of water and ultrasound for 16 h. Next, the initial formed suspension was centrifuged at 6000 rpm to remove the residual unexfoliated g-C_3N_4. Finally, the supernatant was collected and concentrated on a rotary evaporator at 60 °C under reduced pressure, resulting in a milk-like suspension. The mass concentration of the g-CNNS suspension was calculated by weighing the dried power from a certain volume of the suspension.

2.3. The preparation of the modified electrode

The gold electrode (2 mm in diameter) was successively polished in turn with 0.3 and 0.05 μm alumina slurry, and then ultrasonically cleaned in water for 5 min. Prior to modification, the gold electrode was electrochemically cleaned by consecutive cyclic voltammetry (CV) in the potential range of 0–1.6 V in 0.5 M H_2SO_4 until stable CV curves were achieved. 10 μL of cDNA (0.1 μM) was dropped onto the gold electrode surface for 1.5 h at room temperature. The uncovered electrode surface was blocked using 1.0 mM MCH for 1 h for the well-aligned DNA monolayer. Next, the electrode was immersed into the solution containing aptamer (1 μM) for 1.5 h at room temperature to obtain dsDNA-modified electrode. During each modification step, the electrodes should be washed with distilled water in order to decrease the nonspecific adsorption.

2.4. The detection of OTA

The prepared DNA-modified electrodes were soaked into the phosphate buffer saline (PBS, 0.1 M, pH 7.4) containing 0.10 M KCl and different concentrations of OTA for 1 h. After rinsed with distilled water, the electrodes were immersed into the 1 mL of 1 mg/mL g-CNNS solutions for 15 min.

All electrochemical measurements were carried out on a CHI 660E electrochemical working station (CH Instruments, Shanghai, China) at room temperature. Above treated gold electrode, platinum wire and Ag/AgCl (saturated with KCl) were used as the working electrode, the counter electrode and reference electrode, respectively, which act as the three-electrode system. For CV detection, the three-electrode system was incubated with 1 mL of 0.1 M PBS (pH = 7.4) degassed O_2 using Ar and scanned from −0.8 to 0 V vs. Ag/AgCl. For electrochemical impedance spectroscopy (EIS) experiments, the three-electrode system was incubated with the solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$, and the amplitude was 5.0 mV with frequency from 10 kHz to 1 Hz.

2.5. Pretreatment of the real samples

Red wines, juices, and corn, as the real samples, were bought from the supermarket. For red wines and juices, they were pre-filtered by 0.2 μm membrane and then diluted to 5-time using 0.1 M PBS (pH = 7.4) containing 0.10 M KCl. And then, OTA standards with different concentrations were spiked into the diluted samples. The corn was treated by the previous literatures [51]. Briefly, 1 g of non-contaminated and finely ground corn samples were first mixed with 10 mL of extraction solvent (methanol: water = 8:2, v/v) containing different concentrations of OTA, and shaken for 2 h at room temperature. After the centrifugation, the extraction was further filtered by 0.2 μm membrane, and diluted to 5-time using 0.1 M PBS (pH = 7.4) containing 0.10 M KCl.

3. Results and discussions

3.1. Characterization of g-CNNS

The morphology and microstructure of g-CNNS are investigated. As shown the transmission electron microscope (TEM) (Fig. 1A), it can be clearly observed that g-CNNS have a lamellar structure of planar thin nanosheets. From the atomic force microscope (AFM) (Fig. 1B), the