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An ultrasensitive and switch-on platform for aflatoxin B_1 detection in peanut based on the fluorescence quenching of graphene oxide-gold nanocomposites

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ABSTRACT

Graphene oxide-gold nanocomposites (GO/AuNCs) were prepared and used as a novel fluorescence quenching platform for ultrasensitive detection of aflatoxin B_1 (AFB_1) coupled with hybridization chain reaction (HCR) amplification. In the designed system, two fluorophore labeled hairpin probes (HP_1/HP_2) were introduced, and the fluorescence signals of them were effectively quenched due to the adsorption on GO/AuNCs. Associate probe (AP) was used for the specific recognition of AFB_1, and the stem-loop structure of it was opened. Meanwhile, the exposed section of AP was utilized as an initiator for the happen of HCR between HP_1 and HP_2, a strong fluorescence signal was obtained due to the formation of long nicked dsDNA duplex and desorbed of them from the surface of GO/AuNCs. Under the optimal conditions, GO/AuNCs displayed 94% of the quenching efficiency to the fluorescent probes, and a detection limit down to 0.03 pg/mL was obtained for AFB_1 detection. In particular, the assay exhibited excellent selectivity for the detection of AFB_1 against other interfering agents that normally coexist with AFB_1 in mildewed agriculture products. Moreover, the assay could realize the detection of AFB_1 effectively in peanut samples.

1. Introduction

Aflatoxins, that produced by Aspergillus flavus and Aspergillus parasiticus during the growth of crops such as corn, peanut, grain and feed stuff, are of the most predominant and toxic mycotoxins. Thereinto, aflatoxin B_1 (AFB_1) is classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). The propagation of AFB_1 in foods may cause serious diseases such as acute cirrhosis, necrosis and carcinoma [1]. Thus, sensitive detection of AFB_1 is of great importance in disease diagnosis. Recently, commonly used methods for the detection of AFB_1 are surface-enhanced raman scattering (SERS) [2], electrochemistry [3–6], photoelectrochemistry [7], fluorescence [8–11], and colorimetry [12–14]. Among which, fluorescence-based method has attracted special attention due to the merits of it including good selectivity, fast analysis and low-cost.

Graphene oxide (GO), as a derivative of graphene (Gr), displays good water dispersibility and biocompatibility, remarkable electronic and thermal properties. In addition, fluorophore labeled single-stranded DNA (ssDNA) probes could adsorb onto the surface of GO, accompanied with the quenching of fluorescence signals. Based on of this, GO has been widely used as a fluorescent platform to develop biosensors for the detection of DNA, RNA, metal ions and protein [15–22]. Compared to GO, GO functionalized by nanoparticles can exhibit unique physical and chemical properties such as ultrasensitive features and excellent catalytic properties [23,24], and can increase the available surface area for analyte binding [25]. Such characteristics make GO-nanoparticles composites have potential application value in the preparation of optical biosensors. Thereinto, GO [26–31] or functionalized GO including magnetic GO [32,33], reduced GO [34–36], and polymer modified GO [37] have been widely used for the preparation of GO-gold nanocomposites (GO/AuNCs) as highly efficient nanomaterials in the preparation of biosensors. However, most of these reports mainly focus on the preparation of electrochemical and surface enhanced raman scattering (SERS) based biosensors. Lack of fluorescence sensing platform that can realize the detection of AFB_1 by using GO/AuNCs as novel fluorescence quenching materials. Thus, developing fluorescence sensors by using GO/AuNCs for AFB_1 detection is highly desirable.

Developing effective methods that possess high sensitivity and low background noise are of great importance for the preparation of biosensors. Recently, many amplification strategies including polymerase chain reaction (PCR) [38,39], rolling-circle amplification (RCA) [40–42], strand-displacement amplification (SDA) [43,44], nuclease

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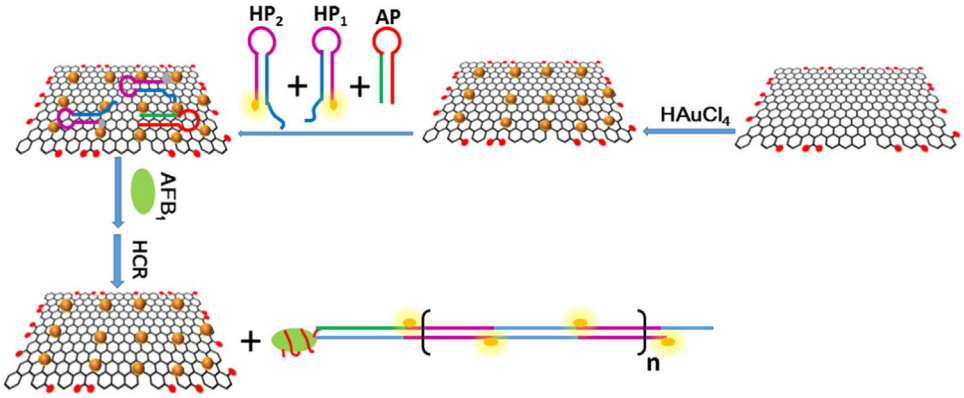

Fig. 1. The schematic illustration of AFB_1 detection based on GO/AuNCs platform and HCR amplification.

assisted target recycling amplification [45–48], and hybridization chain reaction (HCR) [49–52] have been widely applied for the development of biosensors. Thereinto, HCR has been proved to be a powerful signal amplification strategy because of the advantages of it including enzyme-free feature, autonomous protocol, low cost and PCR-like sensitivity, which can achieve by the self-assembly of two stable DNA hairpins, and the signal molecules can attach on the helices of HCR products with precisely controlled density, accordingly improving the efficiency of signal amplification.

Inspired by these developments, an ultrasensitive and switch-on aptasensor was developed herein for AFB_1 detection by using GO/AuNCs as a novel fluorescence quenching platform coupled with HCR signal amplification. The present work contained associate probe (AP) and two fluorophore labeled hairpin probes (HP_1/HP_2). As shown in Fig. 1, AP, HP_1 and HP_2 could adsorb onto the surface of GO/AuNCs in the absence of AFB_1, and the fluorescence signals of them were efficiently quenched. In the presence of AFB_1, AP was used for the specific recognition of AFB_1 and the stem-loop structure of it could be opened, the exposed section of AP was then used as an initiator to happen HCR between HP_1 and HP_2, a strong fluorescence signal obtained due to the formation of long nicked dsDNA duplex and the desorption of them from GO/AuNCs surface. Based on of this, AFB_1 could be quantitatively detected by monitoring the increase of the fluorescence signal.

2. Experimental section

2.1. Materials

Chloroauric acid ($HAuCl_4$), sodium citrate aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), ochratoxin A (OTA), fumonisin B_1 (FB_1) and deoxynivalenol (DON) were purchased from Aladdin Biotech CO. Ltd. (Beijing, China). Graphene oxide was purchased from Nanjing XFNano Mstar Technology. Ltd. (Nanjing, China). 10 mM of phosphate buffer saline (PBS, pH 7.0) was used for the dissolution of DNA strands and the detection of AFB_1. Ultrapure water was provided by a LabWater-EASYQ-A system and used as a solvent in the experiments. All other chemicals were analytical grade and used without further purification. All of the oligonucleotides were synthesized from Sangon Biotech (Shanghai, China) and the sequences of them were shown in Table 1. HP_1 and HP_2 samples were separately heated to 95 °C for 5 min and then gradually cooled to room temperature for future uses.

2.2. Apparatus

Fluorescence spectra were carried out using a Hitachi F-4600 fluorescence spectrophotometer (Hitachi, Japan). The microstructures of GO and GO/AuNCs were carried out using a Hitachi S-8010 scanning electron microscope (SEM, Hitachi, Japan). X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific, Escalab 250Xi spectrometer) was applied to illustrate the disperse states of GO and GO/AuNCs. UV–vis absorption spectra of GO and GO/AuNCs were carried out on an evolution 60 spectrophotometer (Thermo Fisher Corporation, USA).

2.3. Preparation of GO/AuNCs

GO/AuNCs were prepared based on the reduction of $HAuCl_4$ with sodium citrate according to the literature method [53]. Typically, 25 mL of $HAuCl_4$ solution (0.48 mM) was mixed with 1.25 mL of GO solution (1 mg/mL). After that, the mixture was stirred for 30 min with bath heating at 80 ° C. Then 470 μL of sodium citrate (85 mM) was added dropwise. After incubating for 60 min, the mixture was centrifuged at 5000 rpm for 15 min, and the resultant composite was washed with distilled water to remove the free gold nanoparticles (AuNPs). The prepared GO/AuNCs were stored in 4 °C before using.

2.4. Gel analysis of HCR

Agarose gel electrophoresis was carried out to confirm the happen of HCR. Firstly, 1.5 wt% agarose gel was prepared and 4.0 μL of different DNA samples (1.0 μM) were loaded into the lanes. After that, the gel electrophoresis was performed by using 1.0 × TAE as running buffer at a constant potential of 78 V for 40 min. After Stains-All staining by EB solution for 15 min, the gels were photographed by using the gel image system.

2.5. Fluorescent detection procedure of AFB_1

In a typical measurement, 30 nM of AP, 150 nM of HP_1/HP_2 (1:1) were added into 40 μg/mL of GO/AuNCs and incubated at room temperature (RT) for 60 min. Subsequently, different concentrations of AFB_1 were mixed with above solution and incubated at RT for 30 min to

Table 1
Sequences of the DNA strands.

Name	Sequence (5′ – 3′)
AP	5′-$GT_2G_3CACGTGT_2GTCTCTCTGTGTCTCGTGC_3T_2CGCTAG_2C_3A_2G_3CACGAGACACAGA$ – 3′
HP_1	5′-$A_2G_3CACGAGACACAGAGC_2TACG_2C_2TCTGTGTCTCGTGC_3T_2$-FAM – 3′
HP_2	5′-$TAG_2CTCTGTGTCTCGTGC_3T_2A_2G_3CACGAGACACAGAG_2C_2G$-FAM – 3′