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Fluorescence method for quickly detecting ochratoxin A in flour and beer using nitrogen doped carbon dots and silver nanoparticles

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ABSTRACT

In this paper, a FRET (Forster resonance energy transfer) based fluorescence method was developed for the quickly detection of ochratoxin A (OTA) in agricultural products (*e.g.*, flour and beer). A highly fluorescent nitrogen doped carbon dots (CD) were served as energy donor, the DNA and MCH (6-mercapto-1-hexanol) modified Ag nanoparticles were served as energy acceptor in the FRET system. OTA can be detected in a concentration range between 10 and 5000 nM, the limit of detection is 8.7 nM. This method has three advantages: (1) an enhanced fluorescent intensity can be acquired by utilizing the nitrogen doped CD synthesized by one-step approach without sophisticated modification of nanoparticles; (2) OTA detection was accomplished quickly (less than 30 min) by using MCH as assistant molecule; (3) an extended OTA detection linear range was acquired, which may facilitate the OTA detection in real agricultural samples, and is helpful for solving food safety problems.

1. Introduction

Ochratoxin A (OTA) is a kind of mycotoxin which is often found in moldy or fermentative agricultural products [1], it is severely harmful to human body, such as renal toxicity, liver toxicity, *et al.*, especially to the people lived in humid and warm environments (*e.g.,* south of Asia and north of Africa) [2,3]. Therefore, it is valuable to sensitively and quickly detect OTA in agricultural and food species. Nowadays, the traditional methods to detect OTA including the chromatography measurement (such as liquid chromatography (LC) or liquid chromatography-mass spectrograph (LC-MS) methods) [4,5] and antibody based enzyme-linked immunosorbent assay (ELISA) [6,7], which play an important role in the detection and control of OTA in many food related species. However, the relative expensive instrument, sophisticated and time-consuming experimental procedures restrict their widely use in OTA detection for food safety control.

Recently, aptamer was getting more and more attention for the small molecules detection, since it is easy to acquire, has relatively low price and stability, has strong affinity with target. Therefore, the aptasensor based colorimetric method [8–13], fluorescent method [14–19] and electrochemical method [20–23] had been widely employed to detect OTA. For example, OTA detection based on the color change of gold nanoparticle solution [10] or chromogenic reaction catalyzed by DNAzyme [9,24] after the addition of OTA; OTA detection based on the specific interaction of OTA with the aptamer on the surface of electrode, which induced the impedance of electrode changed a lot [21,25]; OTA detection based on the fluorescence signal recovery of fluorescent carbon nanoparticles from aggregated state to dispersed state [26]. These methods have many advantages, such as easy to conduct, without complicated operation, avoid using expensive antibody and many sophisticated instruments. However, some improvements are still needed for these methods; the main purposes are as follows: save the detection time, extend the detection linear range, increase the accuracy of the detection, and simplify the experimental procedures, enhance the detection signal, *et al.* New functional materials and smart experimental design are needed to fulfill these requirements. Recently, carbon dot (CD) had got great attention as it can be easily prepared and functionalized with different functional groups (*i.e.*, -COOH and-NH_2), has stable fluorescent property, therefore, it has been widely used in fluorescent assay for detecting various compounds [12,13,27–30]. Especially, the distance-dependent fluorescent quenching property makes carbon dots suitable for constructing FRET (Forster resonance energy transfer) systems, to effectively enhance the sensitivity of the detection [31]. Recently, Guo *et. al.* had used DNA

Abbreviations: MCH, 6-mercapto-1-hexanol; OTA, ochratoxin A; FRET, Forster resonance energy transfer; LC, liquid chromatography; LC-MS, liquid chromatography-mass spectrograph; ELISA, enzyme-linked immunosorbent assay; CD, carbon dots; AgNPs, silver nanoparticles; cDNA, complementary DNA; Apt-AgNPs, aptamer functionalized AgNPs; cDNA-CD, complementary DNA functionalized carbon dots; TEM, transmission electron microscopy; XPS, X-ray photoelectron spectroscopy; FTIR, Fourier transform infrared; HRTEM, high resolution TEM

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functionalized CD to sensitively detect OTA, the fluorescent intensity of CDs deceased dramatically when they formed aggregates, while after the addition of OTA, the aggregates were broken, accompanied with the fluorescent signal of CD solution enhanced, consequently [26]. Although this method shows high sensitivity, the detection time (more than 2 h) should be shortened, the detection linear range (0–1 ng/mL) should be extended, to facilitate the quickly detection of real samples with different concentrations of OTA.

In this experiment, we would like to develop a method to quickly detect OTA in agricultural products with enhanced sensitivity and wide detection range. Meanwhile, by using aptamer as a versatile platform, FRET as a common approach, we hope this method can also be used for other small molecules detection. In this method, a FRET system contained silver nanoparticles (AgNPs) and CD was proposed based on the hybridization reaction between two DNA strands (aptamer and complementary DNA (cDNA)) on the two probes, respectively. The fluorescent intensity of carbon dots was decreased as a result of the approaching of CD and AgNPs, while the addition of OTA made the fluorescent signal of CD solutions recovered, consequently. In this system, a higher fluorescent intensity can be obtained using nitrogen doped carbon dots; a shorter interaction time was achieved by using MCH (6-mercapto-1-hexanol) as the assistant molecule, as well as the design of tail-to-tail DNA hybridization patterns to decrease the steric hindrance; a wider detection linear range was obtained, which may originate from the strong interaction of aptamer and cDNA. These advantages would facilitate the detection of real agricultural samples contained diverse concentrations of OTA.

2. Experimental section

2.1. Chemical and materials

$AgNO_3$, MCH, ammonium citrate, 1-Ethyl-3-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Adamas Reagent Co., Ltd. (Shanghai, China); Tris-hydroxymethyl aminomethane (Tris), ascorbic acid, trisodium citrate, citric acid, kanamycin sulfate, sisomicin sulfate, tobramycin sulfate, spectinomycin sulfate and acetamiprid were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Zearalenone and aflatoxin M1 were obtained from Sigma-Aldrich Co., Ltd. (USA). OTA was purchased from Pribolab Inc. (Singapore), OTA aptamer [26] (5′-GATCG GGTGT GGGTG GCGTA AAGGG AGCAT CGGAC ACGCC ACCCA CACA-C_6H_{12}-SH-3′) and cDNA (5′-CCT TTA CGC CAC CCA CAC CCG ATC-C_6H_{12}-NH_2-3′) were obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). The flour and beer samples were purchased from a local supermarket. ELISA kit for OTA was obtained from CusaBio Biotechnology Co. Ltd. (Wuhan, China) and was used according to the manufacturer's instruction. All other chemicals were of analytical grade and used without further purification. Double distilled water (dd H_2O) was used throughout the experiment.

2.2. Apparatus

The UV–visible (UV–vis) absorption spectra were recorded on a Tecan Infinite 200 PRO microplate reader (Mannedorf, Swiss). pH values of the solutions were adjusted using a Leici PHS-3E pH meter (INESA Scientific Instrument Co., Ltd., China). The centrifugation process was operated using a CF-10 centrifuge (WiseSpin Co., Ltd., Korea). All measurements were carried out at room temperature otherwise mentioned specially.

2.3. Synthesis of aptamer functionalized AgNPs (Apt-AgNPs)

AgNPs were synthesized according to previous report with minor modification [32] and stored at 4 °C. Briefly, 5 mg ascorbic acid and 40 mg trisodium citrate were mixed in 48 mL H_2O, the pH of the solution was adjusted using NaOH to 11, then, 2 mL $AgNO_3$ (10 mg) was added dropwise on ice-water bath. After stirred for 30 min, the solution was transferred into a 100 °C water bath for 1 h. Then, 3 mL as prepared AgNPs (the concentration of AgNPs is calculated as 120 μg/mL based on previous report [32]) were centrifugated, and the supernatant was discarded, then 1 mL AgNPs was added into the precipitate to acquire a concentrated solution, 50 μL thiolated aptamer (10 μM) and 125 μL Tris-HCl (10 mM, pH 7.4) were added into this solution, and reacted for 24 h at room temperature; then equal volume of NaCl (50 mM) was slowly added into this solution, and put onto a TU-100C digital dry bath incubator (Bluepard, Co. Ltd, China) to slowly evaporate water until the final volume reached at 1/4 of the original solution. Then, Tris-HCl solution (10 mM, pH 7.4) was added to make the final volume is 1 mL. 50 μL MCH (10 mM) was added into this solution afterwards (the high concentration of MCH are sufficient for Ag decoration based on the amounts of MCH, aptamer and AgNPs [32]) and reacted for 30 min at room temperature. The final mixture was centrifuged (13,500 r/min) for 30 min to remove the excess aptamer and MCH, the precipitate was washed with 1 mL Tris-HCl for 2 times, and dispersed into 1 mL Tris-HCl and stored at 4 °C for the future use.

2.4. Synthesis of functional fluorescence CD (cDNA-CD)

The carbon dots were synthesized using citrate acid and ammonium citrate as the precursor according to previous reports with minor modification, respectively [33,34]. Briefly, 0.5 g ammonium citrate or citrate acid was added into 15 mL H_2O, and heated to 200 °C for 30 min, then, the pH value of the solution was adjusted to 8.0 using NaOH. Then 50 μL (4 M) EDC and 50 μL (4 M) NHS were added into 5 mL as prepared carbon dots without further purification (ammonium citrate as the precursor, here the solutions are not purified as the carbon dots are homogeneous according to the microscopic results) and mixed sufficiently, then sonicated for 15 min, 125 μL (10 μM) cDNA was added into this solution and reacted for 40 min at room temperature, and centrifugated at 13,500 r/min for 1 h to remove the unreacted cDNA, and dispersed into the PBS (10 mM, pH 7.4, 50 mM NaCl) for the future use.

2.5. X-ray photoelectron spectroscopy (XPS) measurement

XPS spectra were collected using an Escalab 250Xi X-ray photoelectron spectroscope (Thermo, UK). Carbon dots solution was dropped on a Si substrate and oven-dried before measurement.

2.6. Transmission electron microscopy (TEM) measurement

The TEM micrographs of carbon dots were obtained by a JEM-2100F transmission electron microscope (JEOS, Japan) operated at an accelerating voltage of 200 kV. And the TEM micrographs of AgNPs were obtained by a Tecnai 12 transmission electron microscope (FEI, Netherlands) operated at an accelerating voltage of 120 kV. Specimens were prepared by putting a droplet of sample solution on a carbon-coated copper mesh grid and dried in the air.

2.7. FTIR measurement

The Fourier transform infrared (FTIR) spectra (4000–650 cm^{-1}) were recorded with a Nicolet IS10 FTIR spectrometer (Thermo Fisher Co., Ltd., USA).

2.8. Detection of OTA in aqueous solutions

50 μL PBS (10 mM, pH 7.4, 50 mM NaCl), 30 μL Ca^{2+} (10 mM) and 100 μL cDNA-CD were added into 200 μL Apt-Ag for 10 min, then different concentrations of OTA were added and reacted for 13 min at room temperature, then, the fluorescent spectra were recorded, the