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Fluorescence resonance energy transfer aptasensor between nanoceria and graphene quantum dots for the determination of ochratoxin A

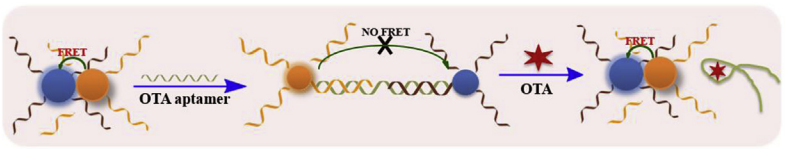
Jiuying Tian, Wenqi Wei, Jiawen Wang, Saijie Ji, Guichan Chen, Jusheng Lu*

Jiangsu Key Laboratory of Green Synthetic Chemistry for Functional Materials, School of Chemistry and Materials Science, Jiangsu Normal University, Xuzhou 221116, PR China

HIGHLIGHTS

- A sensitive ratiometric fluorescent aptasensor for OTA based on FRET.
- Systematic research of the FRET mechanism from nanoceria to GQD.
- Controllable recovery/interruption of FRET from nanoceria to GQD.
- OTA biosensing with wider linear range of 0.01–20 ng mL^{-1} and lower LOD of 2.5 pg mL^{-1}.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present work, colloidal cerium oxide nanoparticles (nanoceria) and graphene quantum dots (GQDs) were firstly synthesized by sol-gel method and pyrolysis respectively, which all have a uniform nano-size and significant fluorescence emission. Due to the fluorescence emission spectrum of nanoceria overlapped the absorption spectrum of GQDs, fluorescence resonance energy transfer (FRET) between nanoceria and GQDs could occur effectively by the electrostatic interaction. Based on it, a sensitive ratiometric fluorescence aptasensor for the determination of ochratoxin A (OTA), a small molecular mycotoxin produced by *Aspergillus* and *Penicillium* strains, has been successfully constructed. In which, probe DNA1@nanoceria and DNA2@GQD were designed to complementary with OTA aptamer, both could adsorb each other, leading to the occur of FRET. After adding of OTA aptamer and then introducing of OTA, the FRET would be interrupted/recovered due to the specific affinity of OTA and its aptamer, the fluorescence recovery value would increase with the addition of OTA. Under the optimal experimental conditions (pH 7, $m_{GQD/nanoceria}$ 2, $c_{aptamer}$ 100 nM, incubation time 30 min), the constructed ratiometric fluorescence aptasensor exhibited a satisfying linear range (0.01–20 ng mL^{-1}), low limit of detection (2.5 pg mL^{-1}) and good selectivity towards OTA, and has been successfully applied for the analysis of real sample peanuts with good accuracy of the recoveries ranged from 90 to 110%.

1. Introduction

Ochratoxin A (OTA) is a mycotoxin as a secondary metabolite derived from various *Aspergillus* and *Penicillium* strains, which is known to cause nephrotoxic, immunotoxic, teratogenic and carcinogenic effects in humans and animals [1,2]. OTA is chemically stable and widely occurs in feed and food chains due to the

* Corresponding author. School of Chemistry and Materials Science, Jiangsu Normal University, 101 Shanghai Road, Xuzhou 221116, PR China.
E-mail address: jushenglu@jsnu.edu.cn (J. Lu).

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contamination of foodstuffs prior to harvest or during storage [3], and European Commission has established a maximum acceptable OTA level of 5 μg kg^{-1} for raw cereal grains [4]. Therefore, it is of considerable significance to develop a rapid and sensitive assay for OTA detection to ensure food safety and avoid the risk of OTA consumption. At present, several analytical methods including high performance liquid chromatography [5,6], electrochemistry [7,8], electrochemiluminescence [9], fluorescence resonance energy transfer (FRET) and aptamer-based biosensing [10–13], have been reported for OTA determination.

Different from antibodies, aptamers are DNA or RNA molecules which can adopt specific three-dimensional conformations to bind target analytes with a high affinity and specificity, which are characterized by high selectivity, high stability, low cost and high thermal stability under a broad array of conditions [14,15]. Aptamers for OTA were selected in 2008 and exhibited high specificity to OTA [16,17], which binding affinities, determined by equilibrium dialysis, were in the nanomolar range [16]. Therefore, many aptasensors combined with fluorescence [11,12,18–20], electrochemical [21,22], surface plasmon resonance [23] and differential pulse voltammetry [24] have been constructed for detecting OTA, and these methods open a new avenue for the detection of OTA.

As a better technique than simple fluorescence, FRET has been widely applied as a sensing mode [25–28], which is a nonradiative phenomenon with the energy transferred from an excited donor fluorophore to a proximal ground-state acceptor through intermolecular dipole-dipole coupling. Generally, the FRET probes have been developed based on the fluorescent quenching or enhancement mechanism. However, such single-wavelength based measurement signal would be easily interfered by various external factors, limiting the accuracy and sensitivity of quantitative determination. Ratiometric FRET strategy, which involves the simultaneous measurement of two fluorescence signals at different wavelengths and use the ratio of the two fluorescence intensities to quantitatively detect the analytes, can alleviate most of the interferences and give greater precision [29–32].

Excellent donor-acceptor pair is a significant factor desired to improve the efficiency of ratiometric FRET and the analytical performance. Graphene quantum dots (GQDs), a new type of zero-dimensional carbon fluorescent nanomaterials [33–36], have superior properties including stable photoluminescence, low cytotoxicity, and high water solubility compared with traditional semiconductor quantum dots containing toxic heavy-metals [37–39], which could be designed as a good fluorescent probe for biosensing. On the other hand, due to the diffusion and reactivity of oxygen vacancies, cerium dioxide nanoparticle (nanoceria) is a unique and promising nanomaterial, in which, the formed oxygen vacancies are associated with a conversion of cerium ions from Ce^{4+} to Ce^{3+} [40]. Under near-UV excitation, excited Ce^{3+} ions to the ground state can emit fluorescence signal, which has successfully applied for a fluorescence probe [41,42].

In the present work, colloidal nanoceria and GQDs were firstly synthesized by sol-gel method and pyrolysis respectively, which all showed excellent fluorescence properties. Based on the mechanism of FRET between nanoceria and GQDs, a sensitive and selective aptasensor for the determination of OTA was constructed. In which, probe DNA1 and DNA2 oligonucleotides were selected to complementary with OTA aptamer, and DNA1@nanoceria and DNA2@GQD were synthesized by chemical bonding. When DNA1@nanoceria mixed with DNA2@GQD, the FRET would occur due to the electrostatic interaction between nanoceria and GQD. Addition of OTA aptamer would limit the FRET because of a large distance between nanoceria and GQD. Furthermore, after introduction of target OTA, FRET from nanoceria to GQD would recover again due to the specific affinity of OTA and its aptamer, which could be applied for quantitative analysis of OTA in real sample.

2. Experimental

2.1. Materials and apparatus

Ochratoxin A (OTA), citric acid, *N*-(3-Dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). OTA aptamer (DNA) was selected according to the literature [16], and its sequence is 5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3'. Probe DNA1 and DNA2 oligonucleotides were complementary to OTA aptamer and their sequences were 3′-NH_2-$(TTT)_3$ CTA GCC CAC ACC-5'and 3′-CTC GTA GCC TGT $(TTT)_3$-NH_2-5′, respectively. All of DNAs were synthesized by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Unless otherwise noted, all chemicals and materials were used as received. Deionized water (18.2 MΩ cm) was obtained from a Milli-Q water purification system and used in all experiments.

The SEM images were recorded with a field emission scanning electron microscopy (FESEM, Hitachi S-4800, Japan). The TEM and HRTEM images were performed on a JEM-2100 transmission electron microscope (JEOL, Japan). UV-vis absorption spectra were measured on a UV-2450 spectrometer (Shimadzu, Japan). Fluorescence spectra were measured on an F-4600 FL spectrophotometer (Hitachi, Japan).

2.2. Synthesis of colloidal nanoceria

The nanoceria was synthesized by sol-gel method and described as follows. Under vigorous stirring, hydrogen peroxide solution (30 vol%) was slowly added into 20 mL of 0.1 M $Ce(NO_3)_3$ aqueous solution dropwise, making the solution from initially colorless to brown and eventually orange. Then concentrated ammonia solution was added rapidly to the above stirred solution, and a lot of blood red precipitate would appear immediately, boiled and cooled to room temperature (RT). After separation, the precipitate was dispersed in water and adjusted the pH to 1–1.5, then heated to reflux until a yellow-green nanoceria hydrosol was generated. Then, 2 mL of polyvinyl alcohol solution (PVA, 10 m/v%) and 2 mL of urea solution (5 m/v%) were added in to the above nanoceria hydrosol, boiled for 40 min, centrifuged, washed with water and ethanol, then dried at 80–85 °C overnight. So, a weakly-agglomerated nanoceria powder was obtained.

2.3. Preparation of GQDs

GQDs solution was prepared from the pyrolyzed citric acid (CA) according to the reported literature with some modification [43]. Briefly, in a round-bottomed flask, 2 g CA was heated at 200 °C for 30 min, and the colorless CA solids turn gradually into orange liquid to gain GQDs. Then, the orange liquid was adjusted to be 7.0 by 10 g L^{-1} NaOH solution under stirring. The obtained GQDs solution was used as stock solution and stored at 4 °C when not used, the concentration was about 2.0 mg mL^{-1}.

2.4. Coupling DNA1 to nanoceria (DNA1@nanoceria) and DNA2 to GQD (DNA2@GQD)

For preparation of DNA1@nanoceria, 0.5 mL of 1.0 μM nanoceria anhydrous ethanol dispersion was added in 2.0 mL of dried dimethyl sulfoxide (DMSO) containing 1.0 mg carbonyl diimidazole at 4 °C for 12 h in the dark, and then centrifuged. The activated nanoceria was added in a 2.0 mL of 10 mM pH 7.0 Tris-HCl buffer

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