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An ultrasensitive aptasensor for Ochratoxin A using hexagonal core/shell upconversion nanoparticles as luminophores

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ABSTRACT

We developed an ultrasensitive luminescence resonance energy transfer (LRET) aptasensor for Ochratoxin A (OTA) detection, using core/shell upconversion nanoparticles (CS-UCNPs) as luminophores. The OTA aptamer was tagged to CS-UCNPs as energy donor and graphene oxide (GO) acted as energy acceptor. The π-π stacking interaction between the aptamer and GO brought CS-UCNPs and GO in close proximity hence initiated the LRET process resulting in quenching of CS-UCNPs luminescence. A linear calibration was obtained between the luminescence intensity and the logarithm of OTA concentration in the range from 0.001 ng mL^{-1} to 250 ng mL^{-1}, with a detection limit of 0.001 ng mL^{-1}. The aptasensor showed good specificity towards OTA in beer samples. The ultrahigh sensitivity and pronounced robustness in beer sample matrix suggested promising prospect of the aptasensor inpractical applications.

1. Introduction

Upconversion nanoparticles (UCNPs) have the excellent ability to combine two or more low energy photons to generate a single high-energy photon by an anti-stokes process (Sun et al., 2015) and hold great promise for a broad range of applications (Zhou et al., 2015; Zhou et al., 2015a), ranging from high-resolution bioimaging to modern photovoltaic technologies (Xu et al., 2015; Yang et al., 2015).

In contrast to conventional luminescent probes, such as QDs (Tani et al., 2014) and fluorescent dyes (Onoe et al., 2014), UCNPs exhibit excellent photostability, high quantum yields, large Stokes shift, continuous emission capability, good chemical stability, and sharp multi-peak line emission (Zheng et al., 2015). However, compared with their bulk counterparts, UCNPs often suffer from much stronger surface quenching effects owing to their high surface-to-volume ratio (Zhou et al., 2015b). Lanthanide-doping processes in these nanoparticles are always accompanied by the trapping of a large portion of dopant ions on the outermost layer of the nanoparticles (Li et al., 2015). Therefore, the luminescence of the surface dopants can be readily quenched by high-energy oscillators arising from surface impurities, ligands, and solvent molecules through multi-phonon relaxation processes. Additionally, the excitation energy carried by the interior ions is likely to be transferred to the surface quenching sites, resulting in non-radiative relaxation (Li et al., 2015; Liu, 2015). To compensate for the deficiencies, growing a shell with small lattice mismatch around the core to reduce non-radiative decay losses of the surface luminescence can provide an effective way to improve the optical properties and the luminescence efficiency of UCNPs (Ding et al., 2015; Wang et al., 2014). In the pioneering work of the researchers, lots of core/shell upconversion nanoparticles (CS-UCNPs) have emerged. Up to now, several groups have successfully fabricated Ln doped α-$NaYF_4$ at α-$NaYF_4$ (Li et al., 2013), β-$NaYF_4$ at α-$NaYF_4$ (Mai et al., 2007) and β-$NaYF_4$ at β-$NaYF_4$ (Wang et al., 2009). These results provide an efficient way to increase the luminescence efficiency of UCNPs, and may be helpful for understanding the mechanism on intensity improvement by coating a passive layer. But these CS-UCNPs are rarely used in the construction of biosensor in food detection. In previously reports, an aptasensor has been constructed for Ochratoxin A (OTA) detection based on UCNPs and AuNRs, which achieved a good result (Dai et al., 2016), but CS-UCNPs are also attempted to synthesize to enhance luminescence efficiency and improve the sensitivity of aptasensor.

OTA belongs to the group of toxic compounds produced as secondary metabolites by several *Aspergillus* and *Penicillium* fungal species (Davis et al., 1969) and is teratogenic, mutagenic, hepatotoxic, nephrotoxic, and immunosuppressive to different animal species

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(Pfohl-Leszkowicz and Manderville, 2007). Various analytical methods have been established for the determination of OTA, based on conventional chromatographic methods, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or gas chromatography (GC) (Garcia-Villanova et al., 2004; Mao et al., 2013; Ventura et al., 2005) coupled to ultraviolet visible, fluorescence, or mass spectrometry. However, these techniques tend to require expensive instrumentation, long processing times, and specially trained personnel. A common alternative for OTA detection is the immunoassay on the basis of antigen-antibody interactions such as enzyme linked immunosorbent assay (ELISA) (Fujii et al., 2007). However, ELISA is a heterogeneous method and needs multiple washing steps; in addition, the antibody is susceptible to environmental and storage conditions, and the ability of the existing antibody-based methods are limited. Instead of using antibody for detection of OTA, aptamer is utilized as molecular recognition agent to build a new sensing platform due to their advantages over antibodies in ease of simple production, easy storage, good reproducibility and particularly target versatility (Li et al., 2014). Aptamers (Kim et al., 2015; Mairal et al., 2008; Yuce et al., 2015) are single stranded oligonucleotides selected in vitro by the systematic evolution of the ligand by the exponential enrichment (SELEX) process from random-sequence nucleic acids libraries.

Graphene oxide (GO) is a derivative of graphene (Wu et al., 2015), equipping with carboxyl groups on the edges, hydroxyl and epoxy groups on the basal plane, which causes the co-existence of the p state from sp^2 carbon clusters and the s state from the sp^3 C-O matrix. This unique heterogeneous electronic structure makes GO to be a super nano-quencher for universal fluorophores, including organic fluorescent dyes, fluorescence proteins, and quantum dots. Therefore, GO has been widely used as an acceptor in luminescence resonance energy transfer (LRET) biosensing using UCNPs as donors or labels studies (Gao et al., 2014). The signal of the labeled component in LRET homogeneous assays is modulated by binding, eliminating the need to separate the bound and the free label. This shortens the total assay time and significantly simplifies the construction of an instrument required to perform an assay automatically (Kuningas et al., 2005). Another reason for choosing GO as energy acceptor is that the aptamer can be easily assembled on its surface through π–π stacking interaction between the nucleobases and the two-dimensional graphitic carbon nanomaterial, such that the covalent label can be omitted (Liu and Zhang, 2015).

In virtue of UCNPs, an ultrasensitive aptasensor for OTA was fabricated based on LRET between CS-UCNPs and GO in this work. The aptamer modified CS-UCNPs could be brought in close proximity to the GO surface based on the strong π-π stacking effect between the nucleobases of aptamer and the sp^2 atoms of GO, resulting in quenching of the luminescence of UCNPs. When aptamer modified CS-UCNPs were incubated with OTA, the aptamers prefer to bind to OTA, which leads to changes in the formation of aptamers and therefore decreases the surface charge of the DNA molecules and the exposure of nucleobases, parts of luminescence of UCNPs was reserved, because the UCNPs are far away from the GO surface. The aptasensor featuring flexible configuration and easy operation provided a detection limit of 0.001 ng mL^{-1} in buffered solutions, which was lowered by orders of magnitude as compared to other reported OTA aptasensors.

2. Material and methods

2.1. Material and reagents

The rare earth chlorides used in this work, including $YCl_3{\cdot}6H_2O$, $YbCl_3{\cdot}6H_2O$, and $ErCl_3$, were of 99.99% purity. Poly(acrylic acid) (PAA, M.W. 2000), 1-octadecene (ODE, 90%), and 2-Morpholinoethanesulfonic Acid (MES) were purchased from Aladdin Industrial Inc. (Shanghai, China). ammonium fluoride (NH_4F), sodium hydroxide (NaOH), cyclohexane, oleic acid (OA), ethanol, Tris·HCl, sodium chloride (NaCl), potassium chloride (KCl), chloroform and calcium chloride ($CaCl_2$) were all of analytical grade. All these chemicals were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Graphene oxide was obtained from Nanjing XFNANO Materials Tech Co., Ltd. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were obtained from Sigma-Aldrich (Saint Louis, MO). Ochratoxin A (OTA) and Ochratoxin B (OTB) were purchased from the Qingdao Pribolab Biological Engineering Co., Ltd. (Qingdao, China). Aflatoxin B1 (AFB1) and Zearalenone (ZEN) were purchased from Sigma-Aldrich (Saint Louis, MO). Fumonisin B1 (FB1) and T-2 toxin (T-2) were obtained from Fermentek, Ltd. (Jerusalem, Israel). The sequences of the OTA aptamer (Cruz-Aguado and Penner, 2008) (5′-biotin-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3′) was synthesized by the Sangon Biotechnology Co., Ltd. (Shanghai, China). All solutions were prepared with ultrapure water (≥18 MΩ, Milli-Q, Millipore).

2.2. Apparatus

The size and morphology of the nanoparticles were determined using a JEM-2100HR transmission electron microscope (TEM, JEOL Ltd., Japan) at 200 kV. X-ray diffraction (XRD) measurements were performed using a D8 Advance instrument (Bruker AXS Ltd., Germany) with graphite-monochromated Cu-Ka radiation (λ=0.15406 nm). The upconversion luminescence spectra were obtained using an F-7000 fluorescence spectrophotometer (Hitachi Co., Japan) modified with an external adjustable continuous-wave 980 nm laser (Beijing Hi-Tech Optoelectronic Co., China) instead of a xenon source. The FT-IR spectra of the bionanoparticles were obtained using a Nicolet Nexus 470 Fourier transform infrared spectrophotometer (Thermo Electron Co., USA) using the KBr method. Ultraviolet-visible (UV–vis) absorption spectra were recorded using a Shimadzu UV-2300 UV–vis spectrophotometer (Shimadzu, Japan). X-ray photoelectron spectroscopic (XPS) measurements were performed on a Thermo Scientific ESCALAB 250Xi (Thermo Scientific Co., U.S.A).

2.3. Synthesis of β-$NaYF_4$:Yb,Er upconversion nanoparticles

The process is similar to Zhang's previously reported (Li and Zhang, 2008; Qian and Zhang, 2008). In brief, $YCl_3{\cdot}6H_2O$, $YbCl_3{\cdot}6H_2O$ and $ErCl_3$ (Ln=80% Y: 18% Yb: 2% Er) were mixed with 6 mL OA and 15 mL ODE in a 100 mL flask, heated to 160 °C to form a homogeneous solution, and then cooled to room temperature. A total of 10 mL of a methanol solution containing 4 mmol of NH_4F and 2.5 mmol of NaOH was slowly added to the flask. The solution was stirred, and the temperature was raised to evaporate the methanol, then the solution was degassed at 100 °C, before being heated to 300 °C and maintained for 1 h under an argon atmosphere. After the solution was cooled, nanocrystals were precipitated from the solution with ethanol and washed with ethanol three times. The core β-$NaYF_4$:Yb,Er UCNPs (core UCNPs) were obtained.

2.4. Synthesis of core/shell β-$NaYF_4$:Yb,Er @$NaYF_4$ upconversion nanoparticles

An amount of $YCl_3{\cdot}6H_2O$ was mixed with 3 mL OA and 17 mL ODE in a 100 mL flask and the solution was heated to 160 °C for 30 min to form a clear yellow solution and then cooled to 80 °C. A solution of 1 mmol of core UCNPs in cyclohexane was added to the solution. After the removal of cyclohexane, a solution of 4 mmol of NH_4F and 2.5 mmol of NaOH in 10 mL of methanol was added, and then the solution was kept at 50 °C for 30 min. After the methanol was evaporated, the solution was heated to 300 °C under argon for 1 h and cooled to room temperature. The core/shell β-$NaYF_4$:Yb,Er @ $NaYF_4$ UCNPs (CS-UCNPs) were collected after centrifugation.