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Magnetic nanobead-based immunoassay for the simultaneous detection of aflatoxin B_1 and ochratoxin A using upconversion nanoparticles as multicolor labels

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ABSTRACT

A novel and sensitive immunoassay for the simultaneous detection of aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) in food samples was developed by using artificial antigen-modified magnetic nanoparticles (MNPs) as immunosensing probes and antibody functionalized upconversion nanoparticles (UCNPs) as signal probes. $NaY_{0.78}F_4$:$Yb_{0.2}$, $Tm_{0.02}$ and $NaY_{0.28}F_4$:$Yb_{0.7}$,$Er_{0.02}$ UCNPs were prepared and functionalized, respectively, with immobilized monoclonal *anti*-AFB_1 antibodies and *anti*-OTA antibodies as signal probes. Based on a competitive immunoassay format, the detection limit for both AFB_1 and OTA under optimal conditions was as low as 0.01 ng mL^{-1}, and the effective detection range was from 0.01 to 10 ng mL^{-1}. The proposed method was successfully applied to measure AFB_1 and OTA in naturally contaminated maize samples and compared to a commercially available ELISA method. The high sensitivity and selectivity of this method is due to the magnetic separation and concentration effect of the MNPs, the high sensitivity of the UCNPs, and the different emission lines of Yb/Tm and Yb/Er doped $NaYF_4$ UCNPs excited by 980 nm laser. Multicolor UCNPs have the potential to be used in other applications for detecting toxins in the field of food safety and other fields.

1. Introduction

At present, nanomaterials-based technology with the multidisciplinary and interdisciplinary characteristics offers great possibilities for the advanced development of new analytical tools and instruments for bioanalytical and biotechnological applications (Zhang et al., 2009).

Conventional downconversion fluorescent materials, including organic dyes (Zhang et al., 2007), semiconductor nanocrystals (such as CdSe, CdTe) (Bruchez et al., 1998), dye-coupled hybrid materials (carbon nanotubes) (Nakayama-Ratchford et al., 2007) and mesoporous silica (Slowing et al., 2007) are fluorophores that are commonly used in biological studies and clinical application because of their unique features. However, they also have many intrinsic limitations (Wang et al., 2006). These downconversion fluorescent materials usually emit one lower-energy photon after absorption of a higher-energy ultraviolet or visible photon. The use of higher-energy light is associated with several significant disadvantages, such as low light-penetration depth, potential severe photodamage to living organisms, and the autofluorescence (noise) of some biological samples (Yi and Chow, 2006). To solve these problems, the development of alternative biological luminescent labels through the use of up-converting rare-earth nanophosphors has attracted a tremendous amount of attention due to the unique luminescence properties of rare-earth nanocrystals. Lanthanide-doped, near-infrared (NIR)-to-visible upconversion nanophosphors are capable of emitting strong visible fluorescence under the excitation of NIR light (typically 980 nm). They have been shown to have significant advantages as fluorescent biolabels over the traditional organic fluorophores due to their attractive optical and chemical features, including low toxicity, large stokes shifts, high resistance to photobleaching, blinking, photochemical stability (Zhang et al., 2006; Lim et al., 2006) and the lack of both auto-fluorescence and a light scattering background (Wang and Liu, 2009). As a result, the signal-to-background ratio and sensitivity of the detection can be greatly improved. Moreover, upconversion nanoparticles have also attracted increasing interest due to their tunable optical properties, made possible by varying the lanthanide dopants (e.g., Tm, Er, Ho) and host matrix used in their synthesis. Based on multicolor fine-tuning in the visible spectral region, they can be applied to simultaneous multiplexed biological labeling. However, to the best of our knowledge, there are few reports related to using upconversion nanophosphors as

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monocolor labels (Wang et al., 2005, 2009a, 2009b; Wu et al., 2011) or for cell imaging (Wang et al., 2009a, 2009b; Hu et al., 2009). Furthermore, there exist even fewer reports dealing with the application of upconversion nanoparticles as multicolor labels in quantitative analysis.

Mycotoxins are a group of chemical substances produced by some fungal species and can cause illness or even death (Santos et al., 2010). There is growing concern regarding mycotoxin contamination in foods and feeds. According to several reports, cereals, olives and dried vines are commodities that could support aflatoxigenic and ochratoxigenic mold growth and aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) production (Molinié et al., 2005). Thus, there is an urgent need for the development of validated analytical methods for the rapid and cost effective screening of mycotoxins at low concentration levels. A wide range of methods are currently available, including thin-layer chromatography, gas chromatography, high-pressure liquid chromatography, fluorescence polarization assays, radioimmunoassays, enzyme-linked immunosorbent assays and fiberoptic based immunoassays (Stroka and Anklam, 2000; Nasir and Jolley, 2002; Shim et al., 2007; Korde et al., 2003; Kolosova et al., 2006; Maragos and Thompson, 1999). Whereas chromatographic methods require extended cleanup steps and derivatization after extraction to get rid of interfering substances, ELISAs require enzymatic reactions, washing and separation of bound and free labels. It is predicted that techniques involving novel nanoparticle labels may become one of the effective approaches for the screening of mycotoxins in the near future.

In the present work, we introduce a simple and sensitive competitive immunoassay for the simultaneous fluorescence detection of two mycotoxins in food samples using AFB_1 and OTA as model analytes. Artificial antigen-modified magnetic nanoparticles (MNPs) were employed as immunosensing probes, and antibody-functionalized upconversion nanoparticles (UCNPs) were used as signal probes; the antibodies-functionalized UCNPs were linked to the surface of the MNPs by antibody–antigen affinity. The method presented here is highly sensitive and selective for the simultaneous detection of AFB_1 and OTA due to the magnetic separation and concentration effect, as well as the high sensitivity of NIR laser-induced upconversion fluorescence without autofluorescence interference. This method has the potential to be applied to the development of novel multiple simultaneous fluorescent detection formats with the advantage of sensitivity and stability over the commercially available ELISA method.

2. Experimental

2.1. Instrument

The size and morphology of nanoparticles were determined using a JEM-2100HR transmission electron microscope (TEM, JEOL Ltd., Japan). X-ray diffraction (XRD) measurements were performed using a D8-advance instrument (Bruker AXS Ltd., Germany). Upconversion fluorescence spectra were measured using an F-7000 fluorescence spectrophotometer (Hitachi Co., U.S.A.) modified with an external 980 nm laser (Beijing Hi-Tech Optoelectronic Co., China) instead of the internal excitation source. Ultraviolet–visible (UV–vis) absorption spectra were recorded using a Shimadzu UV-2300 UV–vis spectrophotometer (Shimadzu, Japan). FT-IR spectra of the bionanoparticles were obtained with a Nicolet Nexus 470 Fourier transform infrared spectrophotometer (Thermo Electron Co., U.S.A.) using the KBr method.

2.2. Reagents

AFB_1 standard solution, OTA standard solution (1 mg mL^{-1} solution in methanol and working dilution by deionized water), AFB_1–BSA antigen (extent of labeling 8–12 mol Aflatoxin B_1 per mol BSA), OTA–BSA antigen (extent of labeling 3–6 mol OTA per mol BSA), monoclonal anti-AFB_1 antibody, and anti-OTA antibody were all purchased from Sigma (U.S.A). 98% 3-aminopropyltrimethoxysilane (APTES) was purchased from Alfa Aesar (U.S.A.).

The rare-earth oxides used in this work, including Y_2O_3, Yb_2O_3, Er_2O_3 and Tm_2O_3 were of 99.99% purity. $FeCl_3{\cdot}6H_2O$, bovine serum albumin (BSA, 96–99%), 25% glutaraldehyde and tetraethyl orthosilicate (TEOS) were of analytical grade. All the chemicals above were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). AFB_1 and OTA ELISA kits were purchased from Jiangsu Suwei Microbiology Research Co. Ltd. (Wuxi, China).

2.3. Synthesis of rare-earth stearate precursors

$NaYF_4$:Yb UCNPs were synthesized according to established methods (Wang et al., 2009a, 2009b; Li et al., 2010) with some modification. To synthesize the ytterbium stearate precursor, 5 mmol Y_2O_3 was dissolved in nitric acid by heating to form yttrium nitrate. Solvent drying resulted in the formation of nitrate powder. In a 500 mL three-neck flask, the as-prepared powder and 30 mmol stearate acid were dissolved in 60 mL of ethanol under vigorous stirring at 50 °C. After the temperature was increased to 78 °C, 30 mmol NaOH in 15 mL of water was added dropwise into the flask over a period of 30 min. The resulting mixture was refluxed at 78 °C for another 30 min. Precipitates from the reaction mixture were filtered and washed three times with water and once with ethanol. The ytterbium stearate precursor was obtained by drying the precipitate at 60 °C for 12 h. Other stearate precursors such as those of erbium, thulium and holmium were prepared in a similar manner employing Yb_2O_3, Er_2O_3 and Tm_2O_3 respectively.

2.4. Synthesis and surface modification of rare-earth-doped $NaYF_4$:Yb upconversion nanoparticles

To synthesize $NaY_{0.28}F_4$:$Yb_{0.7}$, $Er_{0.02}$ UCNPs, 10 mL of water, 15 mL of ethanol, and 5 mL of oleic acid were mixed together while stirring to form a homogeneous solution, to which 0.28 mmol of yttrium stearate, 0.7 mmol of ytterbium stearate, 0.02 mmol of erbium stearate, and 5 mmol of NaF were added. The mixture was stirred for about 15 min, transferred to a 50 mL autoclave, sealed, and treated solvothermally at 195 °C for 24 h. After the autoclave was allowed to cool to room temperature, the precipitates were separated by centrifugation, washed with ethanol three times, and then dried for 12 h. Other rare-earth doped $NaY_{0.78}F_4$:$Yb_{0.2}$, $Tm_{0.02}$ UCNPs were synthesized in a similar manner by varying the amount of rare-earth stearate precursors. Surface modification of $NaYF_4$:Yb, Er/Tm UCNPs was completed by using a typical Stöber-based method (Stöber and Fink, 1968). The details of this preparation are shown in Supplementary section.

2.5. Preparation of amine-functionalized Fe_3O_4 magnetic nanoparticles

Amine-functionalized Fe_3O_4 MNPs were prepared according to Wang and Li's report (Wang et al., 2006). This procedure is illustrated in Supplementary section.

2.6. Preparation of immunosensing probes and signal probes

The artificial antigen conjugated MNPs immunosensing probes were fabricated with the classical glutaraldehyde method (Hun and Zhang, 2007). Typically, 10 mg of MNPs were dispersed in 5 mL of 1.0×10^{-2} M phosphate buffer solution (pH 7.4) by ultrasonication for 20 min. 1.25 mL of 25% glutaraldehyde and 100 mg of $NaBH_4$