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Aptamer biosensor for *Salmonella typhimurium* detection based on luminescence energy transfer from Mn^{2+}-doped $NaYF_4$:Yb, Tm upconverting nanoparticles to gold nanorods

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ABSTRACT

A highly sensitive luminescent bioassay for the detection of *Salmonella typhimurium* was fabricated using Mn^{2+}-doped $NaYF_4$:Yb,Tm upconversion nanoparticles (UCNPs) as the donor and gold nanorods (Au NRs) as the acceptor and utilizing an energy transfer (LET) system. Mn^{2+}-doped $NaYF_4$:Yb,Tm UCNPs with a strong emission peak at 807 nm were obtained by changing the doped ion ratio. Carboxyl-terminated Mn^{2+}-doped $NaYF_4$:Yb,Tm UCNPs were coupled with *S. typhimurium* aptamers, which were employed to capture and concentrate *S. typhimurium*. The electrostatic interactions shorten the distance between the negatively charged donor and the positively charged acceptor, which results in luminescence quenching. The added *S. typhimurium* leads to the restoration of luminescence due to the formation of UCNPs-aptamers-*S. typhimurium*, which repels the UCNPs-aptamers from the Au NRs. The LET system does not occur because of the nonexistence of the luminescence emission band of Mn^{2+}-doped $NaYF_4$:Yb,Tm UCNPs, which had large spectral overlap with the absorption band of Au NRs. Under optimal conditions, the linear range of detecting *S. typhimurium* was 12 to 5×10^5 cfu/mL ($R = 0.99$). The limit of detection for *S. typhimurium* was as low as 11 cfu/mL in an aqueous buffer. The measurement of *S. typhimurium* in milk samples was satisfied in accordance with the plate-counting method, suggesting that the proposed method was of practical value in the application of food security.

1. Introduction

At present, conventional meat and dairy producer contaminations and food poisoning caused by *Salmonella* have become serious issues of food safety that jeopardize human health [1]. With the problem of growing food safety concerns, there is a need to establish simple, efficient, rapid, and sensitive detection methods for *Salmonella* [2]. The methods for detecting *Salmonella* include traditional detection methods, immunological methods [3] and polymerase chain reaction (PCR) [4], However, although the traditional detection method results are accurate and reliable, the cycle is long, complicated to operate, and unable to meet the rapid detection needs. The reagents of the immunological method are costly and prone to false positive signals. Although the PCR sensitivity is good, the method in the extraction of nucleic acid samples is susceptible to pollution. PCR conditions requiring multiple groups for the desired result can be achieved, and the different bases can-not be discriminated between conspecific strains of different serotypes due to the length of the PCR, so the specificity is poor.

Rare-earth-doped upconversion nanoparticles (UCNPs) are capable of emitting strong visible fluorescence excited by low power continuous wavelength lasers as the excitation source (typically 980 nm) [5]. UCNPs have shown outstanding luminescent properties as fluorescent biolabels over the traditional organic fluorophores [6]. The signal-to-background ratio can be greatly improved because of the low toxicity, narrow emission peaks, high resistance to photobleaching, large Stokes shifts, low autofluorescence background [7] and high chemical stability [8,9]. Furthermore, to achieve high photoluminescence efficiency, Mn^{2+}-doped $NaYF_4$:Yb,Tm UCNPs have been prepared by a hydrothermal method [10]. In addition, the carboxyl-functionalized UCNPs serve as the luminescence donor. Gold nanorods (Au NRs) have several advantages, such as good biocompatibility, low toxicity and no photo-induced damage or light interference [11]. These properties point to the strong potential of gold nanorods in the LET system for use as acceptors for NIR range bioassays.

Aptamers, which have high affinity and specificity, are single-stranded DNA or RNA sequence fragments, that can be obtained through a vitro systematic evolution of ligands by exponential enrichment (SELET) [12]. Aptamers bind to target molecules with similar affinity and specificity as antibodies [13], and they provide a variety of advantages over antibodies because of their chemical stability [14],

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ease of synthesis and modification [15,16], inexpensiveness and minimal immunogenicity [17,18]. Based on these advantages [19], aptamers have been designed as electrochemical [20], fluorescent [21] and colorimetric [22] sensors for a wide range of targets [23].

Herein, we describe the design of a turn-on luminescence energy transfer system, in which Mn^{2+}-doped $NaYF_4$:Yb,Tm UCNPs immobilized aptamers onto the surface as the energy donor and Au NRs acted as the energy acceptor. The electrostatic interactions shorten the distance between the negatively charged donor and the positively charged acceptor, which results in luminescence quenching [24]. The added *S. typhimurium* leads to restoration of luminescence due to the formation of UCNPs-aptamer-*S. typhimurium*, which keeps the UCNPs-aptamer away from the Au NRs. This method has a low detection limit and good selectivity, and can detect its targeted bacteria in a short time. The method may promote the application of UCNPs in food safety analysis, especially for the detection of pathogenic bacteria, toxins and viruses.

2. Experimental

2.1. Chemicals and materials

$MnCl_2 \cdot 4H_2O$, $HAuCl_4 \cdot 3H_2O$, $AgNO_3$, $NaBH_4$, and thulium nitrate (99.95%) were purchased from Aladdin Reagent Corporation (Shanghai, China). Ytterbium (III) chloride hexahydrate and yttrium nitrate hexahydrate were purchased from Jinan Henghua Science and Technology Co. Ltd. (China). Poly(acrylic acid) (PAA), 4-(2-hydroxyethyl)-1-piperazi-neethanesulfonic acid (HEPES), 2-(N-morpholino) ethanesulfonic acid (MES), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), diethylene glycol (DEG) (99 + %), *N*-hydroxysuccinimide (NHS), and cetyltrimethyl ammonium bromide (CTAB) were obtained from Sigma-Aldrich (U.S.A.). Sodium citrate, toluene, ethanol, NaF and concentrated hydrochloric acid were purchased from Sinopharm Chemical Reagent Co. (China). *S. typhimurium* aptamers were synthesized by and ascorbic acid was acquired from Shanghai Sangon Biological Science and Technology Company (Shanghai, China). The sequence of the *S. typhimurium* aptamer was 5′-NH_2-GCA ATG GTA CGG TAC TTC CTC GGC ACG TTC TCA GTA GCG CTC GCT GGT CAT CCC ACA GCT ACG TCA AAA GTG CAC GCT ACT TTG CTA A-3′. The strains of *S. typhimurium* were obtained from Wuhu Institute for Food and Drug Control (China). All of the reagents were analytical grade and were used without any further purification. All solutions were prepared with ultrapure water.

2.2. Instruments and characterizations

Transmission electron microscopy (TEM) was performed using a JEOL model 2010 HR instrument operating at 200 kV accelerating voltage to investigate the size and morphology of nanomaterials. The crystal structure was analyzed with a Rigaku RU-200b X-ray powder diffractometer (XRD) with nickel-filtered Cu Kα radiation (λ = 0.14518 nm) 20° ≤ 2θ ≤ 80°. Upconversion nanoparticle fluorescence spectra were measured on a Hitachi F-4600 fluorescence spectrophotometer, which was attached to an external 980 nm laser (Beijing Hi-Tech Optoelectronic Co., China) instead of an internal excitation source. Ultraviolet–visible (UV–vis) absorption spectra were recorded using a Hitachi UV–4100 spectrophotometer. The zeta potential was measured using a Malvern Zetasizer nano ZS90 apparatus (Malvern Instruments, Malvern, United Kingdom).

2.3. Procedures

2.3.1. Synthesis and surface modification of Mn^{2+}-doped $NaYF_4$:Yb^{3+}, Tm^{3+} UCNPs

Mn^{2+}-doped $NaYF_4$:Yb^{3+}, Tm^{3+} UCNPs were prepared according to our previously reported procedure with minor alteration [25]. Briefly, $Y(NO_3)_3$ (0.2 M, 1.2 mL), $MnCl_2 \cdot 4H_2O$ (1.2 M, 145 μL), $YbCl_3$ (0.1 M, 1 mL), $Tm(NO_3)_3$ (0.1 M, 100 μL), and sodium citrate (0.1 M, 1.75 mL) were mixed with ultrapure water (2.1 mL) in a beaker. Then, ethanol (15 mL) and CTAB (0.1 g) were added under magnetic stirring to form a homogeneous solution. Subsequently, aqueous NaF (1.0 M, 6 mL) was added dropwise. The mixed solution was stirred for 2 h. Concentrated nitric acid (1 mL) was added to obtain the complex precursor solution. The solution was transferred into a 50 mL Teflon-lined autoclave and treated at 180 °C for 4 h. The system was allowed to cool to room-temperature naturally. The products were deposited at the bottom of the vessel, were collected by centrifugation at 10,000 rpm for 5 min, and washed once with anhydrous ethanol and three times with deionized water.

Surface modification of Mn^{2+}-doped $NaYF_4$:Yb^{3+}, Tm^{3+} UCNPs was completed using a ligand exchange method [26]. A mixture of PAA (0.2 g) and DEG (16 mL) was added to a four-necked flask and was vigorously stirred under a protective nitrogen flow, and the flask was heated to 110 °C. In quick succession, a toluene solution containing the Mn^{2+}-doped $NaYF_4$:Yb^{3+}, Tm^{3+} UCNPs (16 mg in 2 mL) was quickly injected into the flask. The system was heated to 150 °C and was maintained for 1.5 h. Then, the resulting solution was naturally cooled to room temperature, and hydrochloric acid (0.1 M, 2 mL) was added. The final products were obtained via centrifugation (10,000 rpm, 10 min) and were washed several times with deionized water.

2.3.2. Preparation of amino aptamer-upconversion nanoparticle conjugates

The procedure for the preparation of oligonucleotide-conjugated UCNPs was adapted from a previously reported method [27]. In brief, 1.6 mg of carboxyl-modified UCNPs was first dissolved in 2 mL of 10 mM MES buffer solution at pH 5.6, and sulfo-NHS (1.1 mg) and

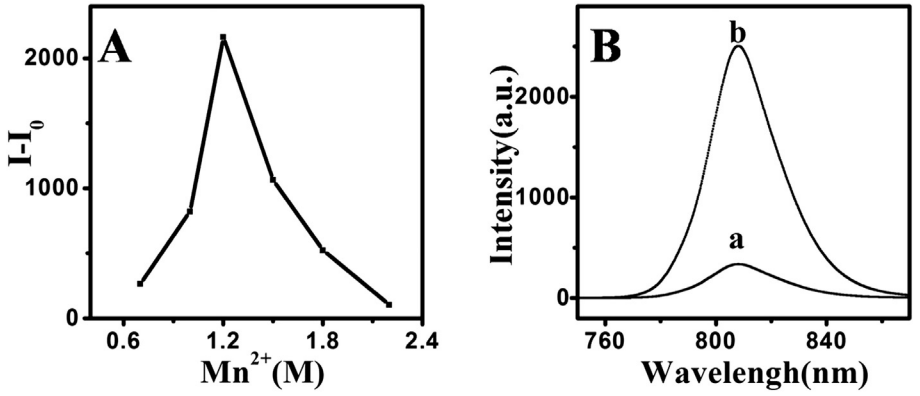

Fig. 1. (A) The effect of added Mn^{2+} on the luminescence intensity of the Mn^{2+}-doped $NaYF_4$:Yb,Tm UCNPs. (B) Emission spectra of (a) the $NaYF_4$:Yb,Tm UCNPs and (b)the $NaYF_4$:Yb,Tm UCNPs with 1.2 M of $MnCl_2 \cdot 4H_2O$.