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Two-color quantum dots-based fluorescence resonance energy transfer for rapid and sensitive detection of *Salmonella* on eggshells

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ABSTRACT

In this paper, we report a novel strategy for highly sensitive, rapid, and selective detection of *Salmonella* Enteritidis using two-color quantum dots based on fluorescence resonance energy transfer (FRET) and fluoroimmunoassay technology. In this FRET system, two-color QDs were adopted as donors and acceptors simultaneously to improve its sensitivity and accuracy. The fluorescence intensity of QDs receptors decreased linearly with the increasing concentrations of *S.* Enteritidis from 75 to 5×10^5 CFU/mL due to FRET system and fluoroimmunoassay reaction. The limit of detection of *S.* Enteritidis in this method was 10 CFU/mL without sample enrichment within 1–2 h. This method has been applied to the analysis *S.* Enteritidis of synthetic samples and eggshell samples. The QDs receptors even can potentially be developed into a sensor for quantitative or qualitative determination of *S.* Enteritidis in the further.

1. Introduction

Salmonellosis is considered one of the most widespread food-borne diseases in the world [1], and Enteritidis is the main serotype responsible for human infections [2–5]. During *Salmonella* Enteritidis (*S.* Enteritidis) contamination and transferation, hen's eggs contaminated are the most important vehicle of the infection [6,7]. Eggs can be contaminated by penetration through the eggshell from the colonized gut or from faeces contaminated during or after oviposition (horizontal transmission) [8,9]. For the faeces contaminated, the effective measures to reduce the *S.* Enteritidis infection on eggshell is to develop cleaned eggs which have been processed by washing, disinfecting under ultraviolet irradiation, drying, coating preservative, and packing before selling [10]. However, cleaned eggs have not been popularized in some developing countries, so the simple, rapid and sensitive detection method of *S.* Enteritidis on eggshell is imperative and important.

Among various S.Enteritidis detection approaches, those based on plate count and enzyme linked immunosorbent assay are frequently used [11,12]. Taqman real-time polymerase chain reaction [13] and flow cytometry detection [14] have also been reported to improve total assay time and further amplification of signals. However, some of these methods are time-consuming, expensive and less sensitive. To cope with these concerns, some researchers are turning to fluorescence analysis in an attempt to overcome these limitations [15,16].

Among fluorescence analyses, the fluorescence resonance energy transfer (FRET) system possesses high sensitivity and selectivity, which has attracted great attentions [17]. FRET occurs when the electronic excitation energy of a donor chromophore is transferred to an acceptor molecule nearby via a through-space dipole–dipole interaction between the donor–acceptor pair [10,18]. It is a powerful technique for probing small changes in the distance between donor and acceptor fluorophores. The FRET process is more efficient when there is an appreciable overlap between the emission spectrum of the donor and the absorption spectrum of acceptor.

Quantum dots (QDs) have been favorably adopted in the FRET-based studies because of large Stokes shift, high quantum yield, good photostability, and size-dependent maximum emission wavelength tunability [19]. FRET-based studies using QDs as the donor are widely applied for bioanalytical chemistry [20], such as nonviral nucleic acid delivery [21], glucose sensing [22], monitoring botulinum neurotoxin A activity [23], optical, and photoelectrochemical sensing [24]. QDs as both the donor and the acceptor are uncommon. However, there are still some studies on the mechanisms of FRET between QDs in aqueous solutions have been studied before confirmed that the QD–QD system perfectly fit the mathematical simulation based on Forster's theory for resonance-energy transfer [25]. QDs have also been used in non-FRET photoluminescence quenching to test its performance [26].

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In this work, we prepared water-soluble QDs with emission maximum located at 514 nm (green-emitting) and 578 nm (orange-emitting), respectively. The green-emitting QDs conjugated with anti-*S.* Enteritidis antibodies (Ab1) as energy donors while conjugating orange-emitting QDs with the secondary antibody for rabbit anti-*S.* Enteritidis antibodies (Ab2) as energy acceptors to establish FRET system. As there are more than one antigenic sites on each bacterium, antibodies can combine the bacterium rapidly which make the assay more sensitive. The design of FRET system incorporating two-color QDs has led to improve signal stability and sensitivity, allowing prolonged measurements, enabling direct detection. The introduction of immunoassay reaction between *S.* Enteritidis and antibody made the selective and rapid capture of *S.* Enteritidis possible. Based on these, the method was used for detection of *S.* Enteritidis from 75 to 5×10^5 CFU/mL within 1–2 h. The limit of detection was 10 CFU/mL. In a word, the union of two-color QDs based on FRET and immunoassay reaction provided a new method for the detection of *S.* Enteritidis. Also, the approach was applicable for the determination of *S.* Enteritidis on egg shells sensitively, fast, and selectively.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade and used without prior purification. Doubly-deionized water (DDW) was used throughout this work. Na_2HPO_4, NaH_2PO_4,*N*-hydroxysuccinimide (NHS), nutrient broth medium (NB), bismuth sulfite agar (BS) were acquired from Guoyao Company (China). 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) was acquired from Aladdin Chemical Reagent Company Ltd. (China). Rabbit anti-*S.* Enteritidis antibody (3–4 mg/mL) (Ab1) and goat anti-rabbit IgG (the secondary antibody for rabbit anti-*S.* Enteritidis antibody) (Ab2) were purchased from Gene Tex-Inc (USA).

Phosphate buffered solution (PBS) with different pH values were prepared by mixing 1/15 mol/L Na_2HPO_4 and 1/15 mol/L NaH_2PO_4 according to certain proportions.

2.2. Apparatus

Absorption spectra were recorded using a Nanodrop 2000c spectrometer (Thermo, USA). Fluorescence spectra were obtained using an RF-5301 spectrophotometer (Shimadzu, Japan). The fluorescence photos under a ZF-6 ultraviolet analyzer (Jiapeng, China) were obtained by using a coolpix S520 digital camera (Nikon, Japan).

2.3. Preparation of water-compatible QDs

Water-soluble and high-luminescent CdTe QDs were synthesized in aqueous solution based on a published method by our laboratory [27]. Briefly, two color QDs, with maximum emission wavelength at 514 nm (green-emitting) and 578 nm (orange-emitting), were used as donor ($QDs_{(D)}$) and acceptor ($QDs_{(A)}$) at the suitable concentration, respectively. The quantum yields (QYs) of these QDs were calculated according to literature [28].

2.4. Optical characterization of $QDs_{(D)}$ and $QDs_{(A)}$

Absorption spectra and fluorescence spectra of $QDs_{(D)}$ and $QDs_{(A)}$ were obtained. The fluorescence intensities were measured at the excitation wavelength of 330 nm. The slit widths of both excitation and emission were 5.0 nm. All optical measurements were carried out at room temperature.

2.5. Conjugating of primary antibodies with $QDs_{(D)}$ and coupling of secondary antibody of $QDs_{(A)}$

The $QDs_{(D)}$ were firstly conjugated with anti-*S.* Enteritidis antibodies by carbodiimide. 200 μL of each of these QDs were activated by adding 100 μL (4 mg/mL) of EDC and 100 μL (0.15 mg/mL) of sulfo-NHS, respectively. The mixed solutions were rotated for 20 min at 37 °C and then, reacted with 200 μL of anti-*S.* Enteritidis antibodies for 2 h at 37 °C. During this process, QDs and antibodies were conjugated through strong covalent bonds. The final bioconjugated QDs were collected and kept at 0–4 °C overnight. The $QDs_{(A)}$ conjugated to Ab2 followed the above procedure.

2.6. FRET between bioconjugated $QDs_{(D)}$ and $QDs_{(A)}$

The fluorescence emission spectra of $QDs_{(D)}$-Ab1, $QDs_{(A)}$-Ab2 and $QDs_{(D)}$-$QDs_{(A)}$ mixture system were measured. In order to further tests FRET phenomenon between the two bioconjugated QDs, the concentration of $QDs_{(A)}$-Ab2 remained the same, while the concentration of $QDs_{(D)}$-Ab1 increased gradually.

2.7. FRET system for detecting S. Enteritidis

The FRET system was built with the green-emitting $QDs_{(D)}$-Ab1 as donor and the orange-emitting $QDs_{(A)}$-Ab2 as receptor. The principle of this protocol was illustrated in Scheme 1. The bio-affinity between Ab1 and Ab2 made these two color QDs close enough to initiate FRET. When higher affinity *S.* Enteritidis added into the $QDs_{(D)}$–$QDs_{(A)}$ system, they occupied the binding sites of the anti-*S.* Enteritidis antibody moiety immunized on $QDs_{(D)}$ due to immunoassay between antigen and antibody. Therefore, the established FRET system was broken and the resonance energy transfer from $QDs_{(D)}$ and $QDs_{(A)}$ was blocked, which released the fluorescence from the $QDs_{(A)}$ and promoted it quenched. In the present case, the immunorecognition of anti-*S.* Enteritidis antibody entailed its specificity and higher affinity for the combination with *S.* Enteritidis. Based on this, it provided a novel FRET approach for *S.* Enteritidis detection.

In practice, in the first step, orange-emitting $QDs_{(A)}$-Ab2 conjugates (4.8×10^{-8} mol/L) were firstly added into the green-emitting $QDs_{(D)}$-Ab1 to facilitate their combination. During this process the fluorescence of green-emitting $QDs_{(D)}$ was quenched owing to the fluorescence resonance energy transfer between the two QDs. In the second step, the *S.* Enteritidis cultures were centrifuged, pouring out of the supernatant and suspending the precipitated *S.* Enteritidis cell in 1 mL of axenic PBS. The process was repeated twice to remove the nutrient medium (NB). At last, the serial 10-fold of *S.* Enteritidis culture dilutions were made in sterile PBS. For quantitative determination, the certain amounts of *S.* Enteritidis culture (75–5×10^5 CFU/mL, respectively) were put into $QDs_{(D)}$–$QDs_{(A)}$ system and kept at 37 °C for 30–40 min. In the last step, *S.* Enteritidis would rapidly and selectively be captured by anti-*S.* Enteritidis antibodies through higher affinity immunorecognition. Then, the combination of the two QDs via the binding site on Ab1 was blocked, and thus, the FRET between them was prohibited. That is, the fluorescence increasing of orange-emitting $QDs_{(A)}$-Ab2 was avoided. The fluorescence intensity of orange-emitting $QDs_{(A)}$-Ab2 was recorded as F_0 and F, in the absence and in the presence of *S.* Enteritidis, respectively. The change of the fluorescence intensity ($\Delta F = F_0 - F$) were used for quantification of *S.* Enteritidis.

2.8. Determination of S. Enteritidis in egg samples by the method of two color QDs based FRET

The egg samples used in the experiment were obtained from a local chicken farm and supermarket. Three types of eggs were used