Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 135 (2015) 428–434

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

The inhibition of fluorescence resonance energy transfer between multicolor quantum dots for rapid and sensitive detection of *Staphylococcus aureus*

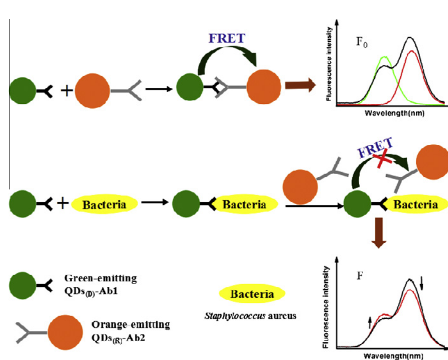
Beibei Wang, Qi Wang, Meihu Ma, Zhaoxia Cai *

National R&D Center for Egg Processing, Food Science and Technology College, Huazhong Agricultural University, 1 Shizishan Street, Wuhan, Hubei 430070, China

HIGHLIGHTS

- A FRET method based on multicolor QDs for *S. aureus* detection are established.
- The two color QDs are used as donors and acceptors in FRET system.
- The fluorescence intensity of QDs quenches linearly with count of *S. aureus* increases.
- The linear ranges are 52 to 2.6×10^5 CFU mL^{-1} with detection limit of 10 CFU/mL.
- This method is satisfactorily applied to the analysis of apple juice and milk samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 June 2014
Received in revised form 7 July 2014
Accepted 15 July 2014
Available online 24 July 2014

Keywords:
Quantum dots
Fluorescence resonance energy transfer
Staphylococcus aureus detection
Food samples

ABSTRACT

In this paper, we constructed the fluorescence resonance energy transfer (FRET) system between two multi-color quantum dots (QDs) of two sizes for rapid and sensitive detection of *Staphylococcus aureus*. In this system, green-emitting QDs conjugated with rabbit anti-*S. aureus* antibodies were used as energy donors while orange-emitting QDs conjugated with goat-anti-rabbit IgG were used as energy acceptors to form FRET system. Pre-binding of *Staphylococcus aureus* (*S. aureus*) on the donor occupied the binding sites and thus blocked resonance energy transfer between two colors QDs, leading to the quenching fluorescence of the acceptor. The fluorescence of acceptor has a linear calibration graph with the concentrations of *S. aureus* from 52 to 2.6×10^5 CFU mL^{-1}. The low detection limit was 10 CFU/mL. It was worth mentioning that the detection method of *S. aureus* had been applied to the analysis of apple juice and milk samples, which could potentially be developed into a sensor in the further study.

© 2014 Elsevier B.V. All rights reserved.

Introduction

Staphylococcus aureus (*S. aureus*) is a commensal and pathogenic bacterium that can cause food-borne disease with a high impact on public health [1–3]. Foods contaminated with *S. aureus* are sometimes implicated in outbreaks of food poisoning due to enterotoxin production by *S. aureus* [4]. Consumption of contaminated food with enterotoxigenic *S. aureus* may lead to *staphylococcal* enterotoxicosis, which is a disease characterized by sudden onset of symptoms including nausea, vomiting, abdominal cramps and diarrhea [5]. Therefore, a rapid detection method for *S. aureus* is of great importance for security purpose.

Traditional taxonomic procedures for identification and classification of *staphylococcal* species usually need enrichment, isolate suspect colonies through selective agar and coagulase test [6]. It

* Corresponding author. Tel.: +86 027 87283177.
E-mail address: caizhaoxia@mail.hzau.edu.cn (Z. Cai).

http://dx.doi.org/10.1016/j.saa.2014.07.024
1386-1425/© 2014 Elsevier B.V. All rights reserved.

tends to be time-consuming and unsuitable for field situations. Other reported methods, such as enzyme-linked immunosorbent assay [7], fourier transform infrared (FTIR) spectroscopy [8], surface plasmon resonance biosensors [9,10] and multiplex PCR [11], realize their excellent performances at the expense of time, cost, and tedious procedures for sample pretreatment or preconcentration. To cope with these concerns, fluorescence analysis based on nanotechnology has been merged to overcome these limitations [12,13].

Fluorescence resonance energy transfer is a nonradiative process whereby an excited state donor (usually a fluorophore) transfers energy to a proximal ground state acceptor through long-range dipole–dipole interactions [14]. Based on the advantages of high detecting speed and sensitivity, FRET system has attracted great attention. In particular, the quantum dots (QDs)-based FRET has been applied to many fields owing to its merit features such as size-tunable spectrum, high quantum yields, broad excitation spectrum, large Stokes shift and good photostability which can avoid the crosstalk caused by spectral overlap of the conventional dye molecules donor and acceptor [15,16]. To date, FRET involving QDs as the donor or acceptor have widely applied in protein interactions [17] or small molecular analysis such as vitamin B_{12} [18], cationic amphiphilic drug [19], glucose [20] and detection of polybrominated biphenyl [21], aflatoxin B_1 [22], *Aspergillus amstelodami* [23] etc.

In this work, we report a binding site pre-blocking approach for the high sensitive detection of *S. aureus* based on the FRET competitive system by using multiple-color water-soluble QDs after attaching to anti-*S. aureus* antibodies (Ab1) or goat anti-rabbit IgG for rabbit anti-*S. aureus* antibodies (Ab2) as energy donor and energy acceptor. Pre-attaching of *S. aureus* with the donors occupied the binding sites for the acceptors and blocked their resonance energy transfer, which led to the fluorescence of acceptor QDs being quenched. This approach avoided the establishment of the equilibrium state for the competitive reaction. Besides, the design of FRET systems incorporating multiple-color QDs as energy donors and acceptors improved signal stability and sensitivity, which allowed prolonged measurements. The introduction of immunoassay reaction between *S. aureus* and antibodies made selective and rapid capture of *S. aureus* possible. Based on these, the method was used for rapid and sensitive detection of *S. aureus* from 52 to 2.6×10^5 CFU/mL within 1 h. In a word, the union of FRET based on multiple-color QDs and immunoassay reaction is providing a sensitive and selective method for the detection of *S. aureus* in food.

Materials and methods

Materials

All reagents were of analytical grade and used without prior purification. Doubly-deionized water (DDW) was used throughout this work. Te powder was acquired from Tianjin Delan Fine Chemical Reagent Company (China). $CdCl_2 \cdot 2.5H_2O$, $NaBH_4$, NaOH, NaH_2PO_4, Na_2HPO_4, thioglycolic acid (TGA), N-hydroxysuccinimide (NHS), Nutrient broth medium (NB), mannitol salt agar (MSA) were acquired from Guoyao Company (China). 1-Thioglycerol (TG) and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) were acquired from Aladdin Chemical Reagent Company Ltd. (China). Rabbit anti-*S. aureus* antibodies (3–4 mg mL^{-1}) (Ab1) and goat anti-rabbit IgG (the secondary antibody for rabbit anti-*S. aureus* antibodies) (Ab2) were purchased from Gene Tex, Inc (USA). Stock cultures of *S. aureus* (ATCC 6538) were obtained from our laboratory culture preservation.

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

Apparatus

Absorption spectra were recorded using a Nanodrop 2000c spectrometer (Thermo, USA). Fluorescence intensity and emission spectra were obtained using an RF-5301 spectrophotometer (Shimadzu, Japan) and 1 cm path-length quartz cuvette was used in experiments. All optical measurements were carried out at room temperature under ambient conditions. All pH measurements were made with a FE20 pH meter (Mettler Toledo, Shanghai).

Preparation of water-soluble QDs of different sizes

Water-soluble and high-luminescent QDs were synthesized in aqueous solution based on a previous published method by our laboratory [24]. Briefly, it was a novel two-step controlled basic water phase method to synthesize size-tunable QDs. With 1-thioglycerol and thioglycolic acid (TGA) as stabilizer, CdTe QDs were prepared by mixing $CdCl_2$ and oxygen-free NaHTe solution under nitrogen protection, where NaHTe were achieved by stirring a mixture of $NaBH_4$ and Te powder at room temperature until black Te powder disappeared. The mixture were heated at 97 °C for 1–2 h to obtain green-emitting QDs with emission maximum at 516 nm. By incubating a mixture of green-emitting QDs and NaOH solution for several hours in the 95 °C water-bath, water-soluble and high-luminescent orange-emitting QDs with emission maximum at 576 nm were prepared. The QDs colloids were then mixed with ethanol at a volume ratio of 1:5 and precipitated under concentration at 8000 rpm for 10 min. The supernatant was discarded and the QDs were blown dried by nitrogen gas and then re-dissolved in PBS buffer (0.01, pH 7.2) to obtain stock solutions of Green QDs and Orange QDs.

The prepared Green QDs and Orange QDs were characterized by spectrometer for absorption spectra and spectrophotometer for fluorescence spectra with the excitation wavelength of 330 nm. The quantum yields (QYs) of these QDs were calculated according to literature [25].

Synthesis of Green QDs–Ab1conjugates and Orange QDs–Ab2 conjugates

To facilitate the reaction between Green QDs and Ab1, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were used as activating agents. 200 μL of Green QDs were activated by adding 100 μL (4 mg mL^{-1}) of EDC and 100 μL (0.15 mg mL^{-1}) of NHS. The mixed solutions were rotated for 20 min at 37 °C and then, reacted with 200 μL of Ab1 for 2 h at 37 °C. During this process, Green QDs and antibodies were conjugated with strong covalent bonds. The unreacted QDs were removed from the media by centrifugal concentrators (Vivaspin 500 centrifugal concentrators, 100 KD). The final Green QDs–Ab1 conjugates were re-dissolved in PBS and kept at 4 °C. The synthesis of Orange QDs–Ab2 conjugates followed the above procedure.

FRET based on Green QDs–Ab1 and Orange QDs–Ab2 conjugates

The fluorescence emission spectra of Green QDs–Ab1, Orange QDs–Ab2 and Green QDs–Ab1/Ab2–Orange QDs FRET system were measured. In order to further research the FRET phenomenon between Green QDs–Ab1 and Orange QDs–Ab2, the concentration of Orange QDs–Ab2 remained the same, while the concentration of Green QDs–Ab1 increased gradually.

FRET system for detecting S. aureus

In practice, at the first step, Orange QDs–Ab2 conjugates (3.0×10^{-8} mol L^{-1}) were firstly added into the Green QDs–Ab1

Download English Version:

https://daneshyari.com/en/article/1233208

Download Persian Version:

https://daneshyari.com/article/1233208

Daneshyari.com