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Ratiometric fluorescence sensor for the sensitive detection of *Bacillus thuringiensis* transgenic sequence based on silica coated supermagnetic nanoparticles and quantum dots

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ABSTRACT

Improving the accuracy and sensitivity of fluorescence analysis is of great importance in clinical diagnosis, environmental and food monitoring. Herein, based on fluorescence resonance energy transfer (FRET), a facile and effective ratiometric fluorescence sensor was constructed for the detection of *Bacillus thuringiensis* (Bt) special gene fragment. In this work, green quantum dots (gQDs) decorated Fe_3O_4 magnetic beads (MBs) with streptavidin (SA) were acted as donor, and gold nanoparticles (GN) modified red quantum dots (rQDs@SiO_2) with hairpin DNA as receptor. When the target sequences exist, the hairpin DNA were unfolded and subsequently captured by MBs@SiO_2@gQD-SA via biotin–SA specific interaction. By using the magnetic separation method, the hybridized composites could be easily purified for florescence tests. By this means, rQDs@SiO_2@GN could enhance the fluorescence intensity of rQDs (I_{620}) and simultaneously quench the fluorescence response of gQDs (I_{540}) via FRET. Under optimal conditions, the ratio of fluorescence intensity at 620 nm and 540 nm (I_{620}/I_{540}) showed that Bt transgene fragment detection owning a good linearity from 5.0 pM to 10 nM with a detection limit of 0.10 pM (S/N = 3). The high selectivity of the probes was also demonstrated using the single-base and three-base mismatch method.

1. Introduction

Bacillus thuringiensis (Bt) transgene, a specific DNA sequence, can produce Bt Cry proteins that can kill insect pets. Thus, it has been widely used in genetically modified (GM) technology to replace the conventional chemical pesticides [1,2]. Up to now, the cultivation of transgenic crops with exogenous Bt gene were commercially available worldwide since Bt maize has been first commercialised [3]. However, due to the possible potential risks in food security and bio-security, the cultivation of GM crops has raised environmental, food and health concerns [4,5]. Therefore, it is extremely important to develop convenient analytical methods for the selective and sensitive detection of Bt transgene in foods and environment.

Recently, many important analytical methods such as polymerase chain reaction (PCR), electrochemistry, electrochemiluminescence (ECL) and fluorescence methods have been widely used in the detection of specific DNA sequences [6–9]. Among these analytic techniques, fluorescent detection method has aroused great interests in food analysis and bioassays due to its high sensitivity and simplicity [10,11]. Numerous fluorescent probes for DNA detection have been constructed on the basis of molecular hybridization [12], enzyme catalysis [13], hydrogen bonding recognition [14] and metal ions coordination [15] and so on. Nevertheless, most of them employ a sole responsive signal for DNA detection, which may suffer from the signal fluctuation caused by variation in detection system and some external factors [16,17]. Fortunately, ratiometric fluorescence probes have the advantages of eliminating most ambiguous interference by its self-referencing of two emission peaks [18,19]. Owing to the enhanced accuracy, ratiometric fluorescence probes have attracted increasing attention in DNA detection [20,21]. But for most probes/sensors, it is a still-challenging issue to detect specific DNA sequence or low abundance gene with accuracy and sensitivity. To address the challenges, a number of novel ratiometric fluorescence sensors have been developed [22–24]. Unfortunately, most of the ratiometric flu-

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orescence probes are confined to the organic dye molecules, which suffer from disadvantages such as poor water solubility, low photo stability and quantum yield and difficult to produce [25,26]. Additionally, the organic dyes are susceptible to photo-bleaching and some of them undergo significant background fluorescence, which can cause a decrease in the sensitivity [27–29]. Therefore, it is crucial to develop signal-amplified ratiometric fluorescence probes for improving the sensitivity and accuracy based on new fluorescence materials.

In recent decades, a few inorganic fluorescence nanomaterials, such as quantum dots [30], carbon dots [31] and metal (gold and silver) nanocluster [32,33], have received increasing interests. Owing to the superior features such as excellent photo-stability, strong fluorescence intensity, easy surface modification, good size uniformity and broad wavelength tunability [34,35], quantum dots (QDs) have been considered as the ideal fluorescent probes [36]. However, one problem is that when QDs are modified or conjugated, their stability and fluorescence will decrease, and the other one is that the coupled probes are difficult to be separated from the detection system [37,38]. It was reported that silica coating could resist the influence of the external environment (pH and high salt concentrations etc.) and retain the optical properties of the original core/shell particles [38–40]. Also, due to its facile chemical processability and good stability in aqueous media, the silica surface can be easily modified to link bioconjugators such as avidin and streptavidine [41,42]. Meanwhile, a large mount of QDs were preserved with the coating of silica shell, which further promoted the signal intensity and stability [37]. Besides, it is well known that magnetic nanoparticles (MBs) have been widely used in biomedical fields, especially for magnetic immobilization and separation [43,44]. Thus, it is meaningful to design a ratiometric fluorescent probe for the sensitive detection of DNA sequence based on silica coated MBs and QDs.

In this work, we prepared the encapsulation of both Fe_3O_4 MBs and QDs with a silica shell to form hybrid materials denoted as MBs@SiO_2@gQDs and rQDs@SiO_2. Based on the two probes, a ratiometric fluorescent sensor was designed for special Bt fragment sequence detection in aqueous solution (Scheme 1). The whole structure include two steps: (1) silica coated MBs and gQDs loaded MB@SiO_2 were prepared as donor and used for convenient separation [42,45] (Scheme 1**A**); (2) silica coated rQDs (rQDs@SiO_2) and GN loaded rQDs@SiO_2 were synthesized as receptor to quench gQDs fluorescence by FRET (Scheme 1**B**). After that, the biotin tailed hairpin DNA was attached onto rQDs@SiO_2. Then MBs@SiO_2@gQDs was conjugated with SA to capture rQD@SiO_2 for convenient separation. In the absence of target DNA (tNDA), only the fluorescence signal of gQDs appeared as the stem-loop structure was closed and the biotin was masked. If the tNDA exists, the loop of hairpin DNA would hybridize with the tNDA and opened the hairpin so as to be captured by MBs@SiO_2@gQDs, which led to the increase of rQDs fluorescence intensity (I_{620}) and the decrease of gQDs fluorescence intensity (I_{540}) (Scheme 1**C**). The ratio of the fluorescence intensity (I_{620}/I_{540}) behaved a good linearity and high sensitivity for Bt transgene detection. Hence, the ratiometric fluorescent sensor is expected to be a useful analytical tool for detection of specific DNA sequences in environmental and food monitoring.

2. Material and methods

2.1. Chemicals and materials

Water and oil CdSe/ZnS QDs (gQDs: 540 nm; rQDs: 620 nm) were purchased from Xingzi (Shanghai) New Material Technology Development Co., Ltd; SA and the oligonucleotides (Table S1) used in the work were purchased from Shanghai sangon Biotechnology Co., Ltd. All chemicals and solvents were of analytical grade and used without further purification. Ultrapure water obtained from Milli-Q, Millipore (18.2 MΩ resistivity) was used throughout the experiment.

2.2. Instrumentation

Photoluminescence (PL) spectra were acquired on Edinburgh FLS920 spectrometer under an excitation of 360 nm; Zeta potential and hydrodynamic size were measured by dynamic light scatting (DLS) using a Malvern Zeta Sizer (Nano-ZS) system. Transmission electron microscope (TEM) images were taken on a JEM-2010FEF transmission electron microscopy at an accelerating voltage of 200 kV.

2.3. Synthesis and modification of MBs@SiO_2@gQDs-SA

To obtain MBs@SiO_2@gQDs composites, MBs@SiO_2 (MS) was first synthesized according to previous work [45,46]. Next, SA and CdSe/ZnS QDs (gQDs: 540 nm) were introduced to obtain MS@gQDs-SA composites. The detailed procedures were provided in Supplementary information.

2.4. Synthesis and functionalization of rQDs@SiO_2@GN-H3

rQDs@SiO_2 and gold nanoparticles (GN) loaded rQDs@SiO_2 were first prepared based on previous work with some changes [47–49]. Then the capture DNA (H3) was attached on the rQDs@SiO_2@GN via Au-S bond to form the rQDs@SiO_2@GN-H3 composites. The final product was stored in PBS buffer solution (pH = 7.4) at 4 °C for further use. The detailed preparation procedures were described in Supplementary information.

2.5. Construction of ratiometric fluorescent sensor

After the two different fluorescence probes were well prepared, the solution of the two probes were mixed together in a PBS buffer medium (pH = 7.4) containing 0.1 mM NaCl and 3.0 mM $MgCl_2$. To realize the aqueous solution detection, tDNA was introduced to the solution. Then the obtained mixture solution were incubated at 40 °C in a dark room for 90 min. Afterwards, the loop of H3 would hybridize with tDNA and opened the hairpin to expose the biotin, which subsequently hybridized with MS@gQDs-SA via biotin–SA specific interaction. The recognition of the two probes led to the increment of rQDs fluorescence intensity (I_{620}) and the decrease of gQDs fluorescence intensity (I_{540}) (Scheme 1**C**). Finally, the excess rQDs@SiO_2@GN-H3 was separated and removed by an external magnetic field and the remaining solution was collected for fluorescent measurements.

3. Results and discussion

3.1. Characterization of materials

UV–vis absorption and FT-IR spectroscopic experiments were first carried out to investigate the MBs-based materials. As depicted in Fig. S1**A**, UV–vis analysis of MBs (curve a) and amino-functionalized MBs@SiO_2 (MS-NH_2) (curve b) revealed that MBs possess a wide and strong absorption peak at 400 nm, which was consistent with previous work [50]. However, after the conjugation of gQDs on the surface of MS-NH_2, the peak at 400 nm was vanished and a new peak of gQDs at 230 nm appeared (curve c and d), suggesting that gQDs were successfully attached on MBs@SiO_2. Meanwhile, FT-IR spectra of all Fe_3O_4-based materials (Fig. S1**B**) showed a characteristic band at 490 cm^{-1}, which was caused by the Fe—O stretching vibration (curve a, b, d). Besides, MS@gQDs