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A novel biosensor based on DNA hybridization for ultrasensitive detection of NOS terminator gene sequences

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ABSTRACT

In this work, we have successfully designed a novel biosensor based on DNA hybridization for ultrasensitive detection of NOS terminator gene sequences (NOSt). This biosensor was synthesized by connecting single-stranded capture DNA (sDNA)-labeled graphene oxide quantum dots (GOQDs) (QDs-sDNA) as fluorescent probe and graphene oxide (GO) as quencher. The detection principle based on hybridization combinations can occur between QDs-sDNA and complementary target DNA; moreover, QDs-sDNA can bind to GO with significantly higher affinity than QDs-dsDNA. In the absence of complementary target DNA, QDs-sDNA was absorbed onto the surface of GO, and the fluorescence of QDs-sDNA was quenched due to fluorescent resonant energy transfer. In the presence of a complementary target DNA, its hybridization with QDs-sDNA formed QDs-dsDNA, which cannot be adsorbed to the GO surface and this leads to reduced quenching. By comparing the fluorescence intensity of QDs-sDNA and QDs-dsDNA in the presence of GO, we can achieve target DNA detection. Thus, rapid, simple, sensitive, efficient, and eco-friendly detection of NOSt was realized. This biosensor had a detection limit of 0.008 nM and a linear range of 0.05–50 nM. Moreover, this sensor can selectivity detect target DNA compared with random and single-base-mismatched sequences, and was successfully applied to the determination target DNA sequences in biological fluids directly. This sensor can be applied to detect other target DNA sequences by simply changing the types of sDNA coupled to the GOQDs.

1. Introduction

Recently, biotechnology advances and increased demand for crop production have led to the rapid development of transgenic technology and popularization of genetically modified organisms (GMOs) [1–3]. However, with the rapid development of the number and diversity of GMOs, safety problems of genetically modified foods, potential ecological pollution problems, and ethical issues have aroused wide spread concern in the community and caused controversy [4,5]. Therefore, sensitive detection of GMOs is important. NOS terminator gene sequences (NOSt), which are derived from the NOS gene of *Agrobacterium tumefaciens*, are often used as an insert for GMOs and a biomarker of transgenic plants [6,7]. Therefore, sensitive NOSt detection for GMO detection is convenient. In the past few years, various methods have been developed for NOSt detection, such as electrochemical detection [6,8,9], gene chip and GLSS [10], dynamic light scattering (DLS) [11], and real-time PCR [12]; however, these methods still have drawbacks. With the increased GMO problems, finding a rapid, simple, sensitive, efficient and eco-friendly method for detecting NOSt is urgent.

Fluorescent resonant energy transfer (FRET) has been proven to be a sensitive and selective method in biological detection [13–17]. FRET is a near-field energy transfer from a fluorescent donorto a fluorescent acceptor within a close proximity [18,19]. Choosing a pair of good donors and receptors is particularly important for improving the efficiency of FRET. Graphene oxide quantum dots (GOQDs), a novel carbon-based nanomaterial [20], have unique properties, such as easy preparation, easy modification, low toxicity, good biocompatibility, and light stability, and have been widely used in bioimaging [21], light-emitting [22], and environmental fields [23]. However, to the best of our knowledge, GOQDs modified as a fluorescent probe combined with FRET, used in biological detection at a very early stage.

GO, is a two-dimensional carbon crystal with one-atom thickness, which is an excellent fluorescent quencher [24–26], has a higher affinity for single-stranded DNAs (sDNAs) than double-stranded DNAs (dsDNAs) [27]. Hence, GO-based fluorescent sensors have been widely used to design sDNA detection based on the mechanism of DNA hybridization [13,28,29]. Using the "postmixing" strategy (let the sDNA probe hybridize with the com-

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plementary target DNA and then add GO) [30,31], given its rapid and sensitive detection, is becoming popular. However, detecting NOSt using this strategy has not been reported.

Taking the advantage of the exceptional properties of GOQDs and GO, as well as easy preparation of the single-stranded capture DNA (sDNA)-labeled GOQDs (QDs-sDNA), we report a DNA-based sensor for detecting NOSt, wherein QDs-sDNA acted as the energy donor and recognition probe and GO acted as the receptor. The mechanism of detection was due to hybrid combinations occurring between QDs-sDNA and target DNA and QDs-sDNA binding to GO with significantly higher affinity than QDs-dsDNA. In the absence of target DNA, GO can absorb QDs-sDNA onto its surface, and the fluorescence of QDs-sDNA is quenched. However, in the presence of target DNA and due to the hybridization between QDs-sDNA and target DNA (forming QDs-dsDNA), the FRET between QDs-dsDNA and GO cannot occur and the degree of fluorescence quenching is greatly reduced. Thus, the fluorescence intensities of the QDs-dsDNA/GO and QDs-sDNA/GO systems are different and the degree of distinction is proportional to the amount of target DNA added. Following this mechanism, target DNA detection can be achieved.

2. Experimental

2.1. Materialsand chemicals

Citric acid (CA), H_2SO_4, $NaNO_3$, $KMnO_4$, NaCl, KCl, sodium citrate, and graphite powder were purchased from Guangfu Chemicals Company (Tianjin, China). 1-*E*thyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC), *N*-hydroxysuccinimide ester (NHS) were purchased from GL Biochem Ltd. (Shanghai, China). Ultrafiltration tube (3 KD) and dialysis bags (MW:1000) were supplied by Union Carbide Co. (USA). SSC (1.5 mM sodium citrate, 0.15 mM NaCl, and pH 7.8) was used as a washing and binding buffer. Ultrapure water (18.2 MΩ) from the United States Milli-Q purification system was used throughout the experiment. All oligonucleotides related to NOSt (Table S1) were synthesized and purified by HPLC in Sangon Biotech (Shanghai) and protected with TE buffer (10 mM Tris-HCl, 1 mM EDTA, and pH 8.0) placed at −20 °C until use.

2.2. Instruments

Morphological structures of QDs were characterized using a Tecnai G2 F20 electron microscope (FEI, USA). Fluorescence measurements were performed using aLS-55 luminescence spectrophotometer. Ultraviolet–visible (UV–vis) absorption spectra were performed on a Cary 300 UV–vis Spectrophotometer (Varian, USA). Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 380 FT-IR spectrometer at 4000–400 cm^{-1} wavelength range.

2.3. Synthesis of GO and GOQDs

The GO in this study was synthesized according to the modified Hummers method [16]. Briefly, 115 ml concentrated H_2SO_4 was added to a beaker and allowed to cool to 0 °C. A total of 5 g graphite powder, 2.5 g $NaNO_3$, and 15 g $KMnO_4$ were slowly added to the beaker. Temperature was kept at 0 °C, and mixture was stirred continuously. After 2 h, mixture was kept in 35 °C water bath and stirred for 30 min. A total of 230 ml distilled water was then added slowly, and temperature was maintained at temperatures not higher than 98 °C. The beaker was placed in an oil bath at 98 °C for 15 min. Enough warm distilled water was then prepared. The mixture turned brown and was diluted to 700 ml with warm water then poured onto 12.5 ml of 30% H_2O_2. Resulting mixture changed from brown to gold. A total of 5% HCl was hot-filtered and poured into the beaker, and filter cake was thoroughly washed GO was then washed with distilled water until pH = 5 and then dried.

GOQDs were synthesized using an easy "bottom-up" method [32]. Briefly, 2 g CA was placed in a 5 ml beaker and heated to 200 °C. During heating, CA was first liquefied. The liquid color changed from colorless to pale yellow and finally turned orange after 30 min. This color transition implied the formation of GOQDs. The obtained orange liquid was dialyzed in a dialysis bag with a 1000 Da molecular weight cut off against deionized water for 48 h to remove excess CA. Then, the solution in the bag was freeze dried to obtain the solid GOQDs.

2.4. Synthesis of QDs-sDNA

The resulting QDs solution (1 mg/ml, 1 ml) was first activated for 2 h in the presence of EDC (20 mM, 1 ml) and NHS (200 mM, 1 ml) at room temperature. Then, 30 μg (1.0 OD) sDNA (MW:8212.5) was added and the mixture was further incubated for 24 h at 4 °C. Thus, the amino groups of the sDNA and carboxyl groups of the QDs covalently bonded. The reaction solution was centrifuged with ultrafiltration tube (3KD) at 8000 rpm for 20 min three times to remove uncoupled QDs and other small molecules.The filtrate was diluted with SSC (pH 7.8) to 15 μg/ml and kept at 4 °C until use.

2.5. Detection of target DNA

QDs-sDNA (15 μg/ml, 1 ml) and a series of different concentrations of target DNA (0–50 nM) were added to the colorimetric tubes (10 ml), mixed thoroughly, and hybridized for 30 min at 37 °C. Then, GO (2 mg/ml, 100 μl) was added into the above mixtures; the mixtures were diluted with SSC (pH 7.8) to 5 ml, shaken evenly, placed at room temperature, and incubated for 10 min. The fluorescence signals of these solutions were measured by a fluorescent spectrophotometer under an excitation wavelength of 365 nm. Slit widths of excitation measured 5 nm and slit widths of emission reached 10 nm. To prevent background interference, the signal output value was calculated from the relative fluorescence intensity F-F_0/F_0; F_0 and F represent the fluorescence intensity when the target DNA is absent and present, respectively.

2.6. Real sample assay

The practicality of the sensor was studied by using the tissue extract of plant *Glycine max* (L.) Merr as the experimental material. DNA was extracted by phenol–chloroform–isoamyl alcohol and washed with ethanol to remove impurities, and the precipitate was dissolved in TE buffer (pH 8.0) and placed at −20 °C until used. The DNA extract was allowed to stand at 95 °C for 10 min, cooled in an ice bath for 5 min, and finally diluted 100 times with SSC (pH 7.8) for detection.

2.7. Probe stability study

To study the stability of the QDs-sDNA probe, we recorded the fluorescence intensity of the new probe and divided the solution into two parts. A part of the solution was subjected to the following experiment: QDs-sDNA (15 μg/ml, 1 ml) and target DNA (0 nM, 10 nM) were added to the colorimetric tubes (10 ml), mixed thoroughly, and hybridized for 30 min at 37 °C. Then, GO (2 mg/ml, 100 μl) was added into the above mixtures; the mixtures were diluted with SSC (pH 7.8) to 5 ml, shaken evenly, placed at room temperature, and incubated for 10 min; moreover, the fluorescence intensity was recorded. The other part of the solution was placed for 2 weeks at 4 °C; the same treatment was performed, and the fluorescence intensity was recorded. Each experiment was repeated thrice.