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DNA-templated fluorescent silver nanoclusters for sensitive detection of pathogenic bacteria based on MNP-DNAzyme-AChE complex

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ABSTRACT

The rapid and sensitive detection of bacteria is of great importance for the field of food safety, water quality and clinical diagnosis. Herein, we present a novel DNA-templated fluorescent silver nanoclusters (DNA-AgNCs) based sensing system integrated with magnetic nanoparticles (MNP)-DNAzyme-AChE complex for detection of pathogenic bacterial using *Escherichia coli* (*E. coli*) as a target bacteria. The platform takes advantage of a DNA-AgNCs based enzyme-responsive fluorescent signal element and a DNAzyme recognition element, providing an ultrasensitive detection limit of 60 cfu mL^{-1}, and good specificity to the target bacteria. Moreover, the platform could be developed into a potentially versatile system by simply altering corresponding DNAzyme as the objects recognition probes.

1. Introduction

Bacterial contamination has been one of the major public issues, as it could pose a serious threat to food safety, water quality and clinical diagnosis. Thus, the rapid and sensitive detection of bacteria is of great importance. Conventional methods for the bacteria detection mainly include plating and culturing assays, and polymerase chain reaction (PCR) [1]. However, time-consuming or complicated procedures and expensive equipment requirement have hindered their further application.

In recent years, the fluorescence-based assay is receiving great attention and becoming a powerful bacterial detection method due to the merits of reliability, need of simple instruments, high detection sensitivity (such as single molecule detection), fast response time, and multiplexed assays using multi-colour dyes [2,3]. Although a number of fluorescent materials such as organic dyes, quantum dots (QDs), and metallic nanoparticles have been widely employed in the biosensing and bioimaging field, there are still a number of issues. For example, organic dyes have poor photostability, semiconductor quantum dots have large physical size and toxicity, and metallic nanoparticles are of low quantum yields.

DNA-templated silver nanoclusters (DNA-AgNCs), as a new class of promising fluorescence nanomaterials, have recently attracted an increasing interest in biological application due to their unique fluorescent properties: excellent photophysical properties, stability, lower toxicity, strong fluorescence emission, and good biocompatibility [4–6]. Many analytical methods have been established based on DNA-AgNCs for detection of biomolecules [7,8] and heavy metal ions [9]. Recently, Zhang et al. developed a fluorescence turn-on assay of acetylcholinesterase (AChE) based on DNA-AgNCs [10]. The fluorescence of 12 polycytosine-templated silver nanoclusters (dC_{12}-AgNCs) could be significantly enhanced due to the reaction of dC_{12}-AgNCs with thiocholine (TCh), which is produced by catalytic hydrolysis of Acetylthiocholine (ATCh) chloride upon the addition of AChE. Inspired by this, we suppose that the combination of AChE and DNA-AgNCs could be an effective and sensitive novel strategy for the development of fluorescent biosensors. However, to fabricate a versatile biosensor for biomolecules, a recognition element is still required.

Herein, we present a novel DNA-AgNCs based fluorescent assay of bacteria utilizing magnetic nanoparticles (MNP)-DNAzyme-AChE (MDA) complex. This platform combines a DNA-AgNCs based enzyme-responsive fluorescent signal element and a DNAzyme recognition element. DNAzymes (also known as deoxyribozymes, DNA enzymes or catalytic DNAs) are catalytically active single-stranded oligonucleotide molecules which can be isolated in vitro from a vast random library method called the systematic evolution of ligands by exponential

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enrichment (SELEX) [11–14]. To date, DNAzymes have been widely used as a recognition element for various biosensors development [15–17]. Recently, Li's group have harnessed this unique property to generate bacteria-specific DNAzyme sensors through in vitro evolution of a vast DNA library against crude extracellular matrix (CEM) components of target bacteria as complex targets [18,19]. In our study, therefore, we decided to use DNAzyme as the recognition element and DNA-AgNCs as a fluorescence signal readout element. Herein, a novel DNA-templated fluorescent silver nanoclusters based sensing system integrated with MDA complex was proposed for detection of pathogenic bacterial using *E. coli* as a target bacteria.

2. Experimental

2.1. Materials and reagents

Streptavidin-coated magnetic nanoparticles were purchased from Life Technologies. T4 DNA ligase, T4 DNA ligase buffer, polynucleotide kinase (PNK), 1 × PNK buffer, acetylcholinesterase (AChE) and sulfo-SMCC were purchased from Sigma-Aldrich Co. (USA). Acetylthiocholine (ATCh) iodide, silver nitrate (99.99%), sodium borohydride ($NaBH_4$ powder, 98%) and all other chemicals are of analytical grade, purchased from Sinopharm Chemical Reagent CO. Ltd (China). UNIQ-10 Spin Column DNA Gel Extraction Kit for PAGE was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). A centrifuge filtration device 3 K cutoff was bought from Amicon bioseparations-Millipore (USA.). Ultrapure water (18.2 MΩ cm) was used in all experiments. All the DNA sequences were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequences were shown in Table 1.

2.2. Preparation of DNAzyme

The *E. coli*-specific DNAzyme used in the study consisting of an enzyme strand and a catalytic substrate strand was generated by template-mediated ligation of substrate strand to enzyme strand [19]. Briefly, 2 nmol of ES were phosphorylated using 10 units (U) of PNK in a 100 μL 1 × PNK buffer (50 mM Tris − HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP) at 37°C for 30 min and the reaction was quenched by heating the mixture at 90 °C for 5 min. Then the mixture was cooled down to room temperature and equimolar CS and TS were added to the reaction mixture. The resultant DNA mixture was then heated to 90°C for 1 min and cooled to room temperature to form double helix structure. Then, 10 U of T4 DNA ligase and 10 μL of 10 × T4 DNA ligase buffer were added and the total volume was adjusted to 100 μL with ddH_2O. The mixture was then incubated at room temperature for 2 h. Finally, the DNA was concentrated by ethanol precipitation and purified by 10% denaturing polyacrylamide gel electrophoresis (dPAGE) and the DNAzyme was finally obtain using Spin Column DNA Gel Extraction Kit for PAGE.

2.3. Preparation of DNA-AChE conjugates

The DNA-AChE conjugates were synthesized according to previously reported protocol with minor modification [20]. Briefly, 1 nmol LS was mixed with 4.3 μL of sulfo-SMCC (20 nmol) and adjusted to a final reaction volume of 100 μL PBS buffer to react at room temperature for 1 h. The excess sulfo-SMCC was removed by a centrifuge filtration device 3 K cutoff. The column was washed with 50 μL PBS buffer 3 times and the DNA was resuspended in 100 μL of 1 × PBS buffer. The AChE solution (1 mL, 3.6 nmol) was then added to the sulfo-SMCC activated DNA (LS). The conjugation reaction was allowed to proceed at room temperature for 1 h. The mixture was filtered through a centrifuge filtration device 100 K cutoff. The DNA-AChE conjugate was then washed with 50 μL PBS buffer 3 times and resuspended in 100 μL buffer. The concentration of the DNA-AChE conjugate was estimated to be 10 μM.

2.4. Preparation of MNP-DNAzyme-AChE (MDA) complex

100 μL MNP(1 mg mL^{-1})was washed with 100 μL binding buffer (20 mM Tris − HCl, pH 8.0, 0.5 M NaCl) for twice and resuspended in 100 μL binding buffer. To separate the supernatant and MNP, a magnet holder was used in the experiments. Then, 100 pmol DNAzyme was added, and the reaction was allowed to proceed for 1 h at room temperature with mild shaking. The obtained MNP-DNAzyme was washed twice with 100 μL binding buffer, while the supernatant was removed. Then, 5 μL 10 μM LS-AChE (150 pmol) and 15 μL 0.5 M NaCl was added to the mixture. And the mixture was heated to 45°C for 2 min, and then cooled to room temperature and incubated for 2 h to allow the MDA complex formed through the DNA hybridization. After the reaction stopped, MDA was magnetically separated and washed twice with 100 μL 1× reaction buffer (1 × RB; 1 mM HEPES, pH 7.4, 150 mM NaCl, 15 mM $MgCl_2$, 0.01% tween 20). Finally, MDA was resuspended in 100 μL 1 × RB and stored at 4 °C before use. The concentration of MDA was estimated to be about 1 μM.

2.5. Synthesis of DNA-templated fluorescent silver nanoclusters

The DNA oligonucleotides ($\mathbf{dC_{12}}$) used in this study was showed in Table 1. DNA-AgNCs were synthesized according to the previous reports [10]. Briefly, a DNA strand was first dissolved in 20 mM sodium phosphate solution (PBS) at pH 7.0. Ag NCs were formed by adding $AgNO_3$ to the DNA solution and placed the complex at 4 °C away from light for 15 min, followed by reduction with $NaBH_4$. The final mixture was kept in the dark at 4 °C for 30 min and then placed in the dark at room temperature for the following assay. The aqueous solution of $NaBH_4$ was freshly prepared by dissolving the $NaBH_4$ powder in water and adding the required volume to the DNA/Ag^+ mixture, followed by vigorous shaking for 1 min. The final concentrations of DNA, $AgNO_3$, and $NaBH_4$ were 20, 120, and 120 μM respectively. Unless otherwise noted, the DNA-Ag NCs was diluted with PBS buffer by 20 times (0.05×) for the further use.

Table 1
SynthesizedOligonucleotides (5′→3′) Used in the Experiments [19].[a]

Name	Labels	Sequence
catalytic substrate (CS)	5'-Biotin, adenine ribonucleotide (rA)	TTTTT TTTTT TTACT CTTCC TAGCT rATGGT TCGAT CAAGA
enzyme strand (ES)	none	GATGT GCGTT GTCGA GACCT GCGAC CGGAA CACTA CACTG TGTGG GGATG GATTT CTTTA CAGTT GTGTG ***TTGAA CGCTG TGTCA AAAAA AAAA***
template strand (TS)	none	GACAA CGCAC ATCTC TTGAT CGAAC C
linker strand (LS)	5'-SH	TTTTT T***TTTT TTTTT TGACA CAGCG TTCAA***
dC12	none	CCCCCCCCCCCC

[a] The bold and italic letters in ES and LS are complementary sequences.

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