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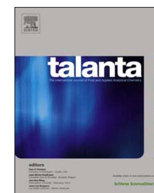

A chemiluminescent aptasensor based on rolling circle amplification and Co^{2+}/N-(aminobutyl)-N-(ethylisoluminol) functional flowerlike gold nanoparticles for *Salmonella typhimurium* detection

Liling Hao[a], Huajie Gu[a], Nuo Duan[a], Shijia Wu[a], Xiaoyuan Ma[a], Yu Xia[a], Haitao Wang[b], Zhouping Wang[a,b,*]

[a] *State Key Laboratory of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China*

[b] *School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China*

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A B S T R A C T

A sensitive steady-state chemiluminescent aptasensor based on rolling circle amplification (RCA) was fabricated for the detection of *Salmonella typhimurium*. The sensor utilized aptamer modified Fe_3O_4 magnetic nanoparticles (MNPs) as capture probes, aptamer as recognition molecules, and Co^{2+} enhanced N-(aminobutyl)-N-(ethylisoluminol) (ABEI) functional flowerlike gold nanoparticles (AuNFs) and complementary strand (cDNA) complex (Co^{2+}/ABEI-AuNFs-cDNA) as signal probes. And P-Iodophenol (PIP) was also added to form a typical ABEI- AuNFs-PIP-H_2O_2 steady-state CL system. By virtue of Fe_3O_4 MNPs based solid-phase RCA strategy, *S. typhimurium* can be first captured by the aptamer immobilized on the surface of Fe_3O_4 MNPs then complex with RCA products to form a sandwich complex. Co^{2+}/ABEI-AuNFs-cDNA signal probes were then assembled on the RCA products to produce and enhance CL signals. Under optimal conditions, the logarithmic correlation between the concentration of *S. typhimurium* and the CL signal was found to be linear within the range of 32 cfu mL^{-1} to 3.2×10^6 cfu mL^{-1} (R^2 =0.9921). The limits of detection of the developed method were found to be 10 cfu mL^{-1} for *S. typhimurium*. The method was also used to detect *S. typhimurium* in real pork samples. The results were compared with those obtained from the plate-counting methods and showed good consistency. Therefore, this detection aptasnesor can be a good candidate for sensitive and selective detection of *S. typhimurium* with simple and effective operations.

1. Introduction

The Gram-negative bacterium *Salmonella typhimurium* (*S. typhimurium*) is one of the most common food borne pathogen for humans and animals. *S. typhimurium* can contaminate various kinds of foods such as pork, egg, milk, fruit juice and so on. And even trace concentrations of bacterial pathogens in food can cause diarrhea, fever, abdominal cramps and affect millions of people annually [1–4]. It is therefore critical to develop rapid, sensitive and selective methods to monitor *S. typhimurium* in food. Nanoparticles based food borne pathogen detection has attracted significant attention. Due to the fascinating aspects such as their good biocompatibility properties, the behavior of the individual particles, size-related electronic, optical properties (quantum size effect), and their applications to catalysis and biology [5–10], nanomaterials are thus playing an increasingly important role in the rapid detection of *S. typhimurium*.

Chemiluminescence (CL) method is one kind of promising methods in food safety detection, because in CL reaction, the energy is produced by a chemical reaction, and excitation is not needed for sample radiation. So the interference of light scattering, source instability and high backgrounds can be avoided [11]. And gold nanoparticles can not only be used as a carrier to couple CL reagents, but also be used as a catalyst to enhance CL intensity [12]. The coupling of gold nanoparticles and CL reagents can combine the unique optical properties, excellent catalytic activity, good biocompatibility and stability of nanoparticles with CL properties of the CL reagents [13,14]. N-(aminobutyl)-N-(ethylisoluminol) (ABEI), as a common CL reagent, has been used for CL functionalized gold nanoparticles. Despite that, the CL intensity of ABEI functionalized gold nanoparticles is still need to be improved. To date, two kinds of bifunctionalized gold

* Corresponding author at: State Key Laboratory of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China.
E-mail address: wangzp@jiangnan.edu.cn (Z. Wang).

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Table 1
DNA sequences used in the work.

Oligonucleotides	Sequences
Aptamer-primer complex	5′-TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAGTTTTTTTGTCCGTGCTAGAAGGAAACAGTTAC-3′
Padlock template	5′-PO_4-TAGCACGGACATATATGATGGTACCGCAGTATGAGTATCTCCTATCACTACTAAGTGGAAGAAATGTAACTGTTTCCTTC-3′
Detection probe	5′-GTTTCCTTCTAGCACTTTTTT-$(CH_2)_6$-SH-3′
Biotinylated aptamer	5′-TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAGTTTTTT-biotin-3′

nanoparticles(BF AuNPs) have been reported by Cui' group [15,16] to enhance the CL intensity, one was prepared by 2-[bis[2-[carboxymethyl-[2-oxo-2-(2-sulfanylethylamino)ethyl]amino]ethyl]amino] acetic acid (DTDTPA) and Co^{2+} to form Co^{2+}/DTDTPA/ABEI-AuNPs complex and the other was prepared by cysteine (Cys) and Cu^{2+} to form Cu^{2+}-Cys/ABEI-AuNPs complex. However, the synthesis of chelator DTDTPA was tedious and time-consuming, and it is hard to immobilize recognized molecules such as aptamer on Cu^{2+}-Cys/ABEI-AuNPs. Therefore, it is critical to develop a simpler, easier and more universal method to enhance the CL intensity.

According to the previous work in our group [17], chitosan and ABEI were used to synthesize ABEI functionalized flowerlike gold nanostructures (ABEI-AuNFs), they can exist on the surface of AuNFs. And chitosan contains chemically active functional groups that and can strongly interact with divalent metal ions through primary amine groups [18]. Besides, Co^{2+} can catalyze the luminol-H_2O_2 CL reaction [19]. Thus, it is naturally to get a simpler, easier and more universal approach to prepare Co^{2+}/ABEI-AuNFs with the purpose of enhancing the CL intensity. Nevertheless, the CL detection can be further improved by combining the novel and powerful signal enhancement methods.

Rolling circle amplification (RCA) is a simple and efficient molecular amplification technique. During the process, long ssDNA with tens to hundreds of tandem repeats can be produced by continuously adding nucleotides (nt) to a primer annealed to a circular template in the presence of polymerase [20–25]. More recently, RCA has attracted significant attention mainly due to the following advantages, first, unlike conventional nucleic-acid amplification reactions such as PCR, this amplification method is achievable at constant temperature with easy operation [26]. Additionally, RCA products contain repetitive sequences that are complementary to the circular DNA template and therefore can be tailor-designed through manipulation of the template, thus the signal molecules can be assembled onto the repetitive sequences which can greatly amplify the detection signal. Among these signal molecules, there are fluorescent dye [27], bio-bar-coded gold nanoparticles [28], quantum dots [29], hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$) [30], luminol [31–33] and so on. To the best of our knowledge, CL reagents functional nanoparticles has not been reported as signal molecules in RCA detection.

Aptamers possess a number of competitive advantages over antibodies including higher specificity, affinity, chemical stability, availability and flexibility. Especially when applying aptamers to solid-phase RCA, aptamers can be conjugated to RCA primers through standard DNA synthesis and therefore eliminate the extra antibody-primer conjugation steps used in antibody based solid-phase RCA [34,35].

Herein, a novel steady-state chemiluminescent aptasensor based on RCA was fabricated for the detection of *S. typhimurium*. By virtue of Fe_3O_4 MNPs based solid-phase RCA strategy, *S. typhimurium* can be first captured by the aptamer immobilized on the surface of Fe_3O_4 MNPs then complex with RCA products to form a sandwich complex. Co^{2+}/ABEI-AuNFs-cDNA signal probes were then assembled on the RCA products to produce and enhance CL signals. To the best of our knowledge, it is the first report to use a steady-state chemiluminescent aptasensor which combines label free Co^{2+}/ABEI-AuNFs-cDNA signal probes with RCA to detect *S. typhimurium* with high sensitivity and specificity.

2. Materials and methods

2.1. Materials and instruments

A 0.1 M stock solution of ABEI was prepared by dissolving ABEI (TCI (Shanghai) Development Co., Ltd, Shanghai, China) in 0.1 M NaOH solution without further purification and stored at 4 °C. A $HAuCl_4$ stock solution (0.2% $HAuCl_4$, w/v) was prepared by dissolving $HAuCl_4 \cdot 4H_2O$ (Shanghai Reagent, Shanghai, China) in purified water and stored at 4 °C. P-Iodophenol (PIP) (purity > 98.0%) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (purity > 98.0%) were purchased from TCI (Shanghai) Development Co., Ltd (Shanghai, China). Chitosan was purchased from Sigma-Aldrich Shanghai Trading Co., Ltd, (Shanghai, China). 25% glutaraldehyde ($OHC(CH_2)_3CHO$), $FeCl_3 \cdot 6H_2O$, NaOH, 1,6-hexanediamine, anhydrous sodium acetate, glycol, ethanol were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China)·H_2O_2 used in detecting buffer were freshly prepared daily from 30% (v/v) H_2O_2 (Xinke Electrochemical Reagent Factory, Bengbu, China). All other reagents used in this study were of analytical grade. The oligonucleotides used in this work (synthesized by Shanghai Sangon Biological Science & Technology Company, Shanghai, China) were shown in Table 1. The meat tested in this work was bought form local market. Ultrapure water was prepared with a Millipore Milli-Q system and was used throughout the study.

Transmission electron microscopy (TEM) was performed using a JEOL model 2100HR instrument operating at 200 kV accelerating voltage (TEM, JEOL Ltd., Japan). Ultraviolet–visible (UV–vis) absorption spectra were recorded using a UV-2300 spectrophotometer (Shimadzu, Japan). FTIR spectra of the amino-functionalized NPs were conducted with a Nicolet Nexus 470 Fourier transform infrared spectrophotometer (Thermo Electron Co., USA) by using the KBr method. The powder X-ray diffraction (XRD) pattern was measured using a D8 Avance instrument (Bruker AXS Ltd., Germany). The electrophoresis experiment was carried out with Western Electrophoresis apparatus (BIO-RAD, USA). The CL measurement was conducted on a static injection CL system consisting of a model MPI-M CL detector and a model multifunctional CL detector (Xi'an Remex Analytical Instrument Co., Ltd.). The graphics program used in this work was Origin Pro 2015.

2.2. Bacterial strains and culture media

S. typhimurium ATCC 14028, *Staphylococcus aureus* (*S. aureus*) ATCC 29213, *Escherichia coli* (*E. coli*) ATCC 25922 were obtained from the American Type Culture Collection (ATCC). *Listeria monocytogenes* (*L. monocytogenes*), *Bacillus cereus* (*B. cereus*) were kindly provided by the Animal, Plant and Food Inspection Centre, Jiangsu Entry-Exit Inspection and Quarantine Bureau (Nanjing, China). The bacteria were grown in Luria-Bertani (LB) (BD Difco) until an OD600 of 0.3 was obtained. The bacteria cells were pelleted at 4000 rpm and 4 °C and then washed twice in 0.01 M phosphate buffer solution (PB) (pH 7.4) at room temperature.

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