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A visual detection method for *Salmonella* Typhimurium based on aptamer recognition and nanogold labeling

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ABSTRACT

A rapid, specific and visible detection method for *Salmonella* Typhimurium was developed based on the recognition of aptamers coupled with nanogold labeling and silver signal amplification. Briefly, biotinylated aptamer 1, which was specific to *Salmonella* Typhimurium, was immobilized onto the surface of microtiter plate-wells modified with streptavidin through the binding of biotin and streptavidin. Then, the target bacteria, *Salmonella* Typhimurium, and the aptamer 2-gold nanoparticle (aptamer 2-AuNPs) conjugates were incubated in the wells to form sandwich-type aptamer/bacteria/aptamer-AuNPs complexes. Under optimal conditions, the correlation between the concentration of *Salmonella* Typhimurium and the intensity of the signals was observed to be linear within the range of $10–10^6$ cfu mL^{-1} ($R^2 = 0.9913$), and the detection limit of the proposed method was observed to be 7 cfu mL^{-1} This developed method offers the potential for rapid, sensitive and visible detection of *Salmonella* Typhimurium in samples.

1. Introduction

Salmonella is the second most frequently reported gastrointestinal zoonotic infection in humans, and it is the primary cause of infectious gastroenteritis outbreaks in the European Union (Arnold, Carrique-Mas, McLaren, & Davies, 2011). Furthermore, *Salmonella* Typhimurium, which is a Gram-negative foodborne pathogen, is recognized as the second most common serotype (after *S.* enteritidis) of *Salmonella* found in humans (Pournaras, Koraki, & Prodromidis, 2008). The rapid, stable and sensitive detection of *Salmonella* Typhimurium is extremely important for food safety. The current primary detection methods for *Salmonella* Typhimurium include traditional culture and flat-counting, Enzyme-Linked Immunosorbent Assay (ELISA), biosensors, electrochemistry and PCR (Kumar, Balakrishna, & Batra, 2008; Lan, Wang, Yin, Clint Hoffmann, & Zheng, 2008; Patela, Bhagwatb, Sanglaya, & Solomon, 2006; Salam & Tothill, 2009). These methods offer various advantages that satisfy different testing requirements, but they also suffer from different disadvantages, such as being time-consuming and possessing poor sensitivity, low stability, high cost and the generation of false positives. The stability and the preparation cost of the antibodies considerably restrict the application of these detection methods, especially the ELISA method.

Aptamers are DNA or RNA molecules that can bind to their target molecules with high affinity and specificity, such as drugs, proteins and other organic or inorganic molecules (Tombelli, Minunni, & Mascini, 2005). Aptamers are commonly obtained in vitro using a combinatorial chemistry technique, which is known as systematic evolution of ligands by exponential enrichment (SELEX) (Hamula, Guthrie, Zhang, Li, & Chris Le, 2006), from a large DNA or RNA library that contains $10^{13}–10^{15}$ different randomized sequences (Osborne & Ellington, 1997). The application of SELEX to synthesize aptamers for a large number of targets has facilitated a wide range of applications that use aptamers. Moreover, because aptamers are stable, inexpensive, simply synthesized and chemically modified, and minimally immunogenic (Duan et al., 2012), aptamers have become useful tools for analytical (Chung, Kim, Jung, & Chung, 2013; Wu, Duan, Wang, & Wang, 2011), diagnostic and therapeutic applications (Keefe, Pai, & Ellington, 2010; Wang, Yang, & Hong, 2011).

Silver-enhancement signal amplification technology has been widely used to improve the detection sensitivity in various analytical applications, such as the detection of pathogens, immunoassays and DNA hybridization assays. In the presence of the reducing agent, the silver ions in solution can be reduced to silver metal. In addition, gold nanoparticles (AuNPs) can catalyze the reduction of the silver ions to silver; therefore, a layer of silver can

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be formed around the AuNPs. In other words, AuNPs are subsequently coated with silver and grow into micrometer-sized particles, which can be easily observed with the naked eye. To date, AuNP labeling and silver enhancement signal amplification technology-based bioassays have been used in a variety of forms for the detection of different targets, such as proteins, amino acids, and nucleic acids (Chen, Huang, & Chang, 2010; Chen, Tang, et al., 2010; Guieua et al., 2011; Li, Chen, Zhao, Guo, & Mu, 2010; Liang et al., 2011; Wang, Chen, Qian, & Zhao, 2009; Zhao et al., 2011). However, there are only a few reports concerning aptamer recognition coupled with AuNP labeling and silver enhancement signal amplification technology (Liu, Ge, & Zhao, 2011; Wang, Wu, et al., 2011; Zhang, Chen, & Zhao, 2009), and even few literature dealing with the whole-cell bacteria detection using aptamer as recognition elements and coupled with silver enhancement signal amplification technology. In this study, we developed a simple and sensitive visual detection method for *Salmonella* Typhimurium using aptamers as recognition and capture elements coupled with AuNP labeling and silver enhancement signal amplification technology. The developed method offers the advantages of stability, sensitivity, and a low cost, and it is easy to read.

2. Materials and methods

2.1. Materials and reagents

Hydroquinone ($C_6H_6O_2$), trisodium citrate dihydrate ($C_6H_5Na_3O_7\cdot 2H_2O$), citric acid monohydrate ($C_7H_8O_7\cdot H_2O$), chloroauric acid tetrahydrate ($HAuCl_4$), sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), sodium nitrate ($NaNO_3$) and sliver nitrate ($AgNO_3$) were of analytical grade, and Arabia gum powder was of practical grade. All the above chemicals were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Streptavidin from Streptomyces avidinii was purchased from the Shanghai Sangon Biological Science & Technology Company (Shanghai, China). The *Salmonella* Typhimurium (*S.* Typhimurium) ATCC 50761 was obtained from the American Type Culture Collection (ATCC). One sequence of the *S.* Typhimurium aptamer (Raghavendra, Harish, Hari, Senthil Kumar, & Jaykus, 2009) was 5′-biotin-C6-TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G-3′ (aptamer 1), and the other sequence of the *S.* Typhimurium aptamer (Raghavendra et al., 2009) was 5′-SH-TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G-3′ (aptamer 2). Both aptamers were synthesized by the Shanghai Sangon Biological Science & Technology Company (Shanghai, China).

2.2. Apparatus

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 Shimadzu UV-2300 UV–vis spectrophotometer(Shimadzu, Japan). The AuNPs solution was centrifuged using a Centrifuge 5430R (5430R, Eppendorf, Germany). The signals were scanned using a Thermo Labsystem MK3 Microplate Reader (MK3, Thermo Labsystem, U.S.A).

2.3. Preparation and functionalization of the AuNPs

AuNPs (approximately 13 nm in diameter) were prepared using the citrate reduction method (Liu & Lu, 2006). The resulting solution of AuNPs was characterized using TEM (JEOL, Japan) and UV–vis spectrophotometry (UV-2300, Shimadzu, Japan), and the results of these measurements are presented in Fig. 1. The concentration of AuNPs was estimated by Beer–Lambert law using extinction coefficient, $2.43 \times 108\ M^{-1}\ cm^{-1}$ at 520 nm. The preparation of aptamer 2-AuNPs was as follows. A total of 1 mL of the AuNPs solution was centrifuged for at least 35 min at 12,000 rpm (5430R, Eppendorf, Germany) to remove the excess reagents, such as trisodium citrate dihydrate, and then 500 μL of distilled water was added after the supernatant was removed. Then, 10 μL of aptamer 2 was added, and the mixture was incubated at 37 °C. After incubation for 24 h, a NaCl solution ($1\ mol\ L^{-1}$) was added after the addition of approximately 5 mg of SDS, and the total volume of the adding NaCl solution was 120 μL. The solution was allowed to age for 24 h, followed by centrifuging for at least 35 min at 12,000 rpm to remove the excess reagents. After removing the supernatant, the red oily precipitate was washed with $0.1\ mol\ L^{-1}$ NaCl and a $10\ mmol\ L^{-1}$ phosphate buffer solution ($0.01\ mol\ L^{-1}$ PBS, pH 7.4), re-centrifuged, and redispersed in $10\ mmol\ L^{-1}$ phosphate buffer solution ($0.01\ mol\ L^{-1}$ PBS, pH 7.4). Subsequently, the aptamer 2-AuNPs was obtained and stored in the dark at 4 °C.

2.4. Analytical procedure

First, 200 μL of Streptavidin was added into each zone of the microtiter plate for 12 h at 4 °C, washed 3 times with $0.01\ mol\ L^{-1}$ PBS and then air dried. Second, 100 μL of 1% BSA was added into each zone of the microtiter plate for 30 min at Room Temperature (RT) to prevent the appearance of a false positive, washed 3 times with $0.01\ mol\ L^{-1}$ PBS and then air dried. Third, 10 μL of aptamer 1 was added into each

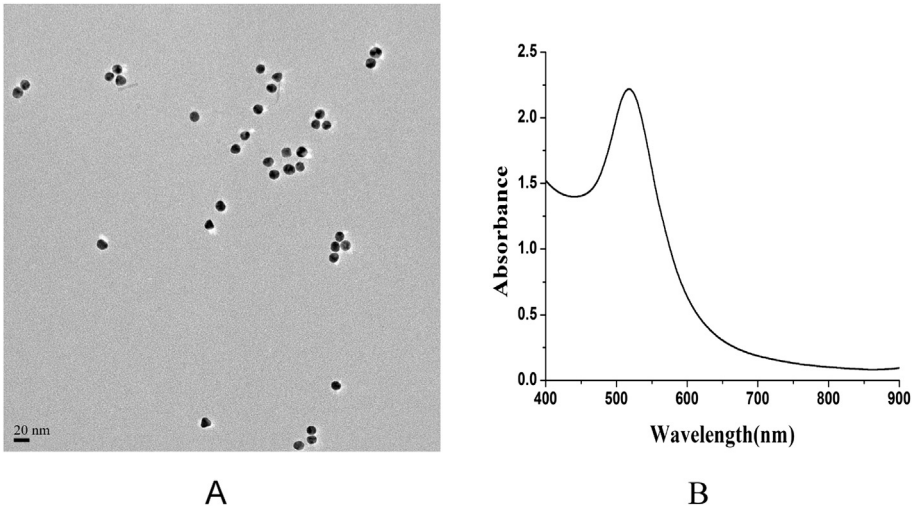

Fig. 1. (A) TEM image of the AuNPs and (B) UV–vis spectrum of the AuNPs.