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A novel aptasensor for the colorimetric detection of *S. typhimurium* based on gold nanoparticles

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ABSTRACT

A simple, fast and convenient colorimetric aptasensor was fabricated for the detection of *Salmonella typhimurium* (*S. typhimurium*) which was based on the color change effect of gold nanoparticles (GNPs). *S. typhimurium* is one of the most common causes of food-associated disease. Aptamers with specific recognition toward *S. typhimurium* was modified to the surface of prepared GNPs. They play a role for the protection of GNPs from aggregation toward high concentrations of NaCl. With the addition of *S. typhimurium*, aptamers preferably combined to *S. typhimurium* and the protection effect was broken. With more *S. typhimurium*, more aptamers detached from GNPs. In such a situation, the exposed GNPs would aggregated to some extent with the addition of NaCl. The color changed from red, purple to blue which could be characterized by UV–Vis spectrophotometer. The absorbance spectra of GNPs redshifted constantly and the intensity ratio of A700/A521 changed regularly. This could be calculated for the basis of quantitative detection of *S. typhimurium* from 10^2 cfu/mL to 10^7 cfu/mL. The obtained linear correlation equation was $y = 0.1946x–0.2800$ ($R^2 = 0.9939$) with a detection limit as low as 56 cfu/mL. This method is simple and rapid, results in high sensitivity and specificity, and can be used to detect actual samples.

1. Introduction

Gold nanoparticles (GNPs) have been widely explored in the field of analytical chemistry, biology, diagnosis and treatment of cancer owing to their specific optical, chemical, electrochemical and catalytical properties. They could combine with a variety of biological macromolecules and will not affect the biological activity. GNPs could be synthesized by several reductants, such as sodium citrate, sodium borohydride, HEPES-NaOH, et al. (Grabar et al., 1996; Phadtare et al., 2003; Xie et al., 2007). In recent years, the application of GNPs for the visual detection has attracted more and more attention. These targets mainly include foodborne pathogens, antibiotics, toxins, metal ions, pesticide residues, illegal food additives and so on (Su et al., 2012; Kalidasan et al., 2013; Kim et al., 2010; Yang et al., 2011; Wu et al., 2013; Xu et al., 2010). Tanga has used GNPs labeling and silver staining method to achieve the visual detection of trachoma Chlamydia (Tanga et al., 2010). The detection limit can reach 2 ng/mL. Shyu has successfully realized the lateral chromatography detection of *Staphylococcus aureus* enterotoxin B using GNPs and silver enhancement technique with a detection limit of 10 pg/mL (Rong-Hwa et al., 2010). Besides, in the treatment of diseases, Nam has designed a kind of "smart" GNPs. The exposure to acidic environment or cancer cells could result in the aggregation of the "smart" GNPs (Nam et al., 2009). The absorption peaks shift to the near infrared wavelength and it can be applied to the photo-thermal cancer therapy.

Aptamers are specific oligonucleotide sequences that bind to the target substance (Tombelli et al., 2005). They are usually screened by SELEX technique from a DNA or RNA library (Hamula et al., 2006). Due to their high specificity and affinity with its corresponding target materials, aptamers are widely applied to the analysis and detection field especially in food security detecting (Suh et al., 2014; Kim et al., 2014; Guo et al., 2014; Luo et al., 2014a, 2014b; Ragavan et al., 2013). Girolamo has fixed the OTA aptamer to the surface of the coupled gel (Girolamo et al., 2011). It has been used as a solid phase extraction column to remove OTA from wheat samples. Bai has proposed an array sensor based on the aptamer to detect kanamycin (Bai et al., 2014). It is fast and without any labeling. Xue has established an electrochemical aptasensor for the detection of bisphenol A in water samples within 30 min (Xue et al., 2013). The detection limit can reach 0.284 pg/mL.

S. typhimurium is a common anaerobic gram-negative bacteria, which is widely distributed in the environment. It is first isolated by

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Salmon since the 1885 cholera epidemic and has been found more than 2500 serum types and variants so far. Almost all of these serum types may be parasitic in the intestines of human body or animals. *S. typhimurium* can be transmitted to humans through contaminated poultry, eggs, milk, fish and meat products. With the increasing awareness of food safety, the design of fast, efficient, simple and specific method for the detection of *S. typhimurium* has become an inevitable trend in the field of food safety and health inspection. The traditional culture method for *S. typhimurium* detection is tedious and time-consuming which includes the sequential steps of pre-enrichment, selective enrichment and selective differential plating (Patel et al., 2006). It is difficult to meet the needs of the current detection of foodborne bacteria. A variety of detection methods have been reported based on the convenient and fast detection demand, such as immunofluorescence detection, immunodiffusion method, enzyme linked immunoassay (ELISA), gene chip techniques, resistance detection method and latex agglutination test, et al. (Falkenhorst et al., 2013; Jain et al., 2012; Ma et al., 2014; Luo et al., 2014a, 2014b).

In this study, a new colorimetric detection of *S. typhimurium* based on GNPs and aptamer is discussed. The specific *S. typhimurium* aptamer was first modified at the surface of prepared GNPs. They protect GNPs from aggregation in high NaCl solution. With the addition of *S. typhimurium*, aptamer preferably connected to *S. typhimurium* and detached from GNPs. Without the protection effect of aptamer, GNPs will be aggregated. The color that changed from red, purple to blue could be observed by naked eyes and characterized by UV–Vis spectrophotometer. *S. typhimurium* could thus be quantified. It is a simple, fast, and convenient method without complex detecting instrument. Moreover, it is expected to be applied in other food safety detection fields.

2. Materials and methods

2.1. Materials

Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), Trisodium citrate ($Na_3C_6H_5O_7$), sodium chloride (NaCl) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The *S. typhimurium* aptamer sequence is 5′ - TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G - 3′ which is synthesized by Shanghai Sangon Biological Science & Technology Company (Shanghai, China). The ultrapure water used in the experiments was prepared using a Millipore Direct-Q® 3 system (Merck Millipore, MA, USA) and had a resistivity of 18.2 MΩ cm.

2.2. Preparation of GNPs

GNPs were prepared with some modifications as described by Grabar et al. (1996). First, 4.2 mL $HAuCl_4 \cdot 4H_2O$ (1%, w/w) and 95.8 mL ultrapure water were added to a flask with three necks. The mixture was heated to boil until 10 min under uniform magnetic stirring with oil bath. Then, 10 mL sodium citrate (1%, w/w) was rapidly injected and reacted for another 15 min. The obtained wine-red solution was GNPs. The resulting GNPs were purified by three times of centrifugation (10,000 rpm, 25 min) and were redispersed in 40 mL of ultrapure water. The GNPs were stored at 4 °C for further use and characterization.

2.3. Preparation of GNPs-aptamer complex (aptasensor)

The *S. typhimurium* aptamer solution was centrifuged at 10,000 rpm for 5 min (4 °C). Then it was diluted to 70 pM by PBS. 1 mL GNPs was added to 100 μL 70 pM *S. typhimurium* aptamer solution and incubated at 37 °C for 30 h. The mixture was centrifuged at 10,000 rpm for 10 min (4 °C) to remove the excessive aptamer. Thus, the GNPs-aptamer complex (aptasensor) was prepared and stored at 4 °C for further use.

2.4. Detection of S. typhimurium based on the aptasensor

100 μL gradient dilutions of *S. typhimurium* (10 cfu/mL, 10^2 cfu/mL, 10^3 cfu/mL, 10^4 cfu/mL, 10^5 cfu/mL, 10^6 cfu/mL, 10^7 cfu/mL, 10^8 cfu/mL) was added to 100 μL aptasensor and incubated at 37 °C for 1 h. Then, 1.5 M NaCl was added and mixed. The resulting solution was characterized by UV–Vis spectrophotometer.

2.5. Recovery experiments for milk sample

In this experiment, the commercial milk was used as realistic samples for recovery experiments in the detection of *S. typhimurium*. Gradient dilutions of *S. typhimurium* were added to the milk sample. Then the aptasensor based detection method was conducted to calculate the detectable amount of *S. typhimurium*. The results were compared with the traditional plate counting method and the recovery rate was calculated.

3. Results and discussion

3.1. Aptasensor based detection for S. typhimurium

The aptasensor based detection process using GNPs for *S. typhimurium* is shown in Fig. 1. Firstly, the *S. typhimurium* specific aptamer was attached to GNPs via the effect of thiol group. When the *S. typhimurium*

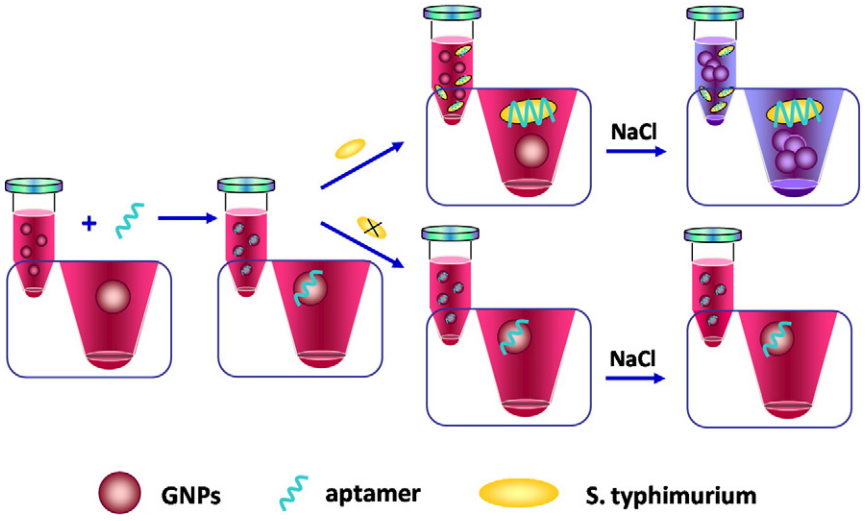

Fig. 1. Schematic illustration for the colorimetric detection of *S. typhimurium* based on GNPs and aptamers.