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Salmonella typhimurium detection using a surface-enhanced Raman scattering-based aptasensor

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ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) has been used in a variety of biological applications due to its high sensitivity and specificity. Here, we report a SERS-based aptasensor approach for quantitative detection of pathogenic bacteria. A SERS substrate bearing Au@Ag core/shell nanoparticles (NPs) is functionalized with aptamer 1 (apt 1) for the capture of target molecules. X-rhodamine (ROX)-modified aptamer 2 (apt 2) is used as recognition element and Raman reporter. *Salmonella typhimurium* specifically interacted with the aptamers to form Au@Ag-apt 1-target-apt 2-ROX sandwich-like complexes. As a result, the concentration of *S. typhimurium* was determined using this developed aptasensor structure, and a calibration curve is obtained in the range of 15 to 1.5×10^6 cfu/mL with a limit of detection of 15 cfu/mL. Our method was successfully applied to real food samples, and the results are consistent with the results obtained using plate counting methods. We believe that the developed method shows potential for the rapid and sensitive detection of pathogenic bacteria in food safety assurance.

1. Introduction

The identification of pathogenic bacteria is an important task in light of the fact that many serious and even fatal medical conditions result from bacterial infection. *Salmonella typhimurium*, a Gram-negative bacteria pathogen, is one of the leading causes of food-borne illness in human and animal hosts worldwide (Brandt et al., 2013). *S. typhimurium* is transmitted primarily through the consumption of raw or uncooked vegetables, poultry, eggs, and fruits (Crum-Cianflone, 2008; Mrema et al., 2006). In China, approximately 80% of food-borne bacteria outbreaks are thought to be caused by *Salmonella* (Yang et al., 2010). This increasing incidence of *S. typhimurium* in different food products is now attracting the attention of the government. The food safety regulations of some countries (e.g., China, USA) require no tolerance of *S. typhimurium* in ready-to-eat food. Thus, sensitive detection methods for *S. typhimurium* play a critical role in many aspects of life, especially in food quality.

The existing common detection methods include culture-based methods, molecular methods of regular and real-time polymerase chain reaction (PCR) (Zheng et al., 2014; Park et al., 2013; Silva et al., 2011), and immunoassays (Mantzila et al., 2008; Shim et al., 2014). Despite many advances in these fields, it is still challenging to find new approaches that could improve the simplicity, selectivity, stability and sensitivity of these analytical methods.

Currently, SERS is suggested as a powerful tool for characterization of chemical and biological analytes due to its high fingerprint information content, its extreme sensitivity and its obliviousness to the aqueous environment intrinsic to biological systems (Chen et al., 2008; Cialla et al., 2012). Since its discovery in the 1970s, SERS has found increasing application in different fields, such as the biomedical field (Haynes et al., 2005; Kaminska et al., 2015), environmental monitoring (Bhandari et al., 2009; Muller et al., 2014), and food quality assurance (Ma et al., 2015; Aoki et al., 2013). Many efforts have been devoted to the SERS detection of bacterial cells (Sundaram et al., 2013a; Banerjee et al., 2010). Among these, some detection methods were designed based on the spectrum of bacteria themselves (Sundaram et al., 2013b). Kowalska et al. (2015) prepared a Cu-based SERS platform for *Staphylococcus aureus* detection. The spectrum of *S. aureus* bacteria exhibits characteristic band vibrations. The band located at 727 cm^{-1} is assigned to the C–N stretching mode of the adenine moiety of the lipid layer in the cell wall and/or to the purine ring breathing mode. The spectral features at 840, 1027, 1037 and 1084 cm^{-1} arise from tyrosine, phenylalanine, C–C oscillations, and phosphate binding in DNA, respectively. Additionally, there are many reports of SERS-based assays that used antibodies as recognition agents (Knauer et al., 2012; Wang et al., 2011). Lin and Hamme (2014) combined monoclonal antibody-conjugated sphere-shaped gold nanoparticles with single-walled carbon nanotubes to create a nanohybrid system to selectively detect *S. typhimurium* DT104 bacteria. The Raman signal intensity was from Rhodamine 6G, with a detection limit of 10^5 cfu/mL. However, the preparation of the antibodies via animal immunization is time-consuming (several months), and the antibodies may become

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susceptible to stability or modification issues. Aptamers with high affinity and selectivity are beginning to emerge as an alternative to antibodies in these applications.

To the best of our knowledge, there have been few reports of using SERS coupled with aptamers for the sensitive and rapid detection of bacteria. Therefore, in the present study, we report a sensitive method for the detection of pathogenic bacteria using a SERS sensing platform based on an aptamer. Our strategy exploits the Au@Ag core/shell NPs as the enhanced substrate for SERS. To selectively detect *S. typhimurium*, we employed apt 1 immobilized on the surface of Au@Ag core/shell NPs. The Raman signal intensity was generated from ROX-modified apt 2, which would bind with the target in the same way as Au@Ag-apt 1. Important factors such as the concentration of Au@Ag-apt 1 and the concentration of ROX-apt 2 were evaluated. The sensitive and selective SERS assay was validated by the analysis of *S. typhimurium* in food samples, showing its great potential for food quality application.

2. Materials and methods

2.1. Materials

Chloroauric acid ($HAuCl_4$), L-ascorbic acid, and $AgNO_3$ were obtained from Sigma-Aldrich (U.S.A.). Trisodium citrate was of analytical grade and was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Milli-Q water with a resistivity of 18.2 MΩ/cm was used throughout the experiments. The *S. typhimurium* aptamers were prepared in our laboratory (Duan et al., 2013) and synthesized and purified by high-performance liquid chromatography (Sangon Biotechnology, Inc., Shanghai, China). The sequences of the *S. typhimurium* aptamers are 5′-SH-AGTAATGCCCGGTAGTTATTCAAAGATGAGTAGGAAAAGA-3′ (apt 1) and 5′-ROX-AGTAATGCCCGGTAGTTATTCAAAGATGAGTAGGAAAAGA-3′ (apt 2).

2.2. Instrumentation

The size and shape of the nanoparticles were tested using transmission electron microscopy (TEM) (TEM, JEOL Ltd., Japan). Absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu Co., Japan). The Raman spectra were measured using a Jobin Yvon micro-Raman spectroscope (Super LabRam II) with a mode of 50 × objective (8 mm), a holographic grating (1800 g/mm), a charge-coupled device detector with 1024 × 256 pixels and a 785 nm excitation laser.

2.3. Bacteria culture

The *S. typhimurium* ATCC 14028 was kindly donated by the Animal, Plant and Food Inspection Centre, Jiangsu Entry-Exit Inspection and Quarantine Bureau (Nanjing, China). *S. typhimurium* was cultivated in Luria-Bertani medium and then incubated in a shaker at 37 °C for 24 h. One hundred microliters of the bacterial culture was diluted with medium and coated on the agar plates and cultured at 37 °C for 18 h to count colony forming units. The rest of the bacteria were harvested by centrifugation at 3000 rpm and 4 °C and washed twice in 1 × binding buffer (1 × BB 50 mM Tris–HCl at pH 7.4, 5 mM KCl, 100 mM NaCl, and 1 mM $MgCl_2$) at room temperature.

2.4. Preparation of Au@Ag core/shell NPs

AuNPs were first synthesized using the citrate reduction method. In brief, all glassware used in the experiment were cleaned with aqua regia (HNO_3/HCl, 3:1, *v*/*v*), rinsed thoroughly in ultrapure water, and dried prior to use. Then, 500 μL of 1% $HAuCl_4$ solution and 49.5 mL Milli-Q water were heated to boiling with vigorously stirring. Then, 1.5 mL of 1% trisodium citrate was rapidly injected into the boiling reaction mixture. After the mixture was boiled for 15 min, the heating source was removed, and the colloid was cooled to room temperature. The prepared Au colloids were used as seeds for the synthesis of Au@Ag core/shell NPs (Olson et al., 2008). A 0.4-mL aliquot of 0.1 M L-ascorbic acid was added to 2 mL of as-prepared AuNPs and stirred for 5 min at room temperature. Then, 1.2 mL of 1 mM $AgNO_3$ was added dropwise and reacted for 1 h under stirring. The solution color changed from wine red to orange during this period. The mixture was centrifuged (10,000 rpm, 15 min) and washed with Milli-Q water three times. The final deposition was suspended in 200 μL of PBS and stored at 4 °C for further use.

2.5. Preparation of aptamer functionalized Au@Ag core/shell NPs

Au@Ag core/shell NPs modified by aptamer were prepared according to the literature with some modification (Wang et al., 2007). The immobilization of aptamer onto Au@Ag core/shell NPs occurs through covalent bonding between Ag and the terminal thiol group. Briefly, 10 μL of 10 μM apt 1 was added to 200 μL of the already prepared Au@Ag core/shell NPs solution and reacted at 4 °C with gentle shaking for 12 h. The Au@Ag-apt 1 complex was then aged with salts (0.1 M NaCl, 10 mM phosphate, pH 7.0) for 40 h. The prepared complex was centrifuged at 12,000 rpm for 15 min twice to remove the free aptamer. The Au@Ag-apt 1 was then dispersed in 100 μL of binding buffer for subsequent experiments.

2.6. SERS analysis of sample

Various concentrations of samples (10 μL) were mixed with 175 μL of Au@Ag-apt 1 complex and allowed to incubate for 45 min at room temperature. Then, 15 μL of ROX-labeled apt 2 (10 μM) was added and incubated for another 45 min. Following incubation, the mixtures were centrifuged at 3000 rpm and 4 °C for 5 min to remove the extra unbound ROX-apt 2, and the precipitated mixtures were washed twice and resuspended in 200 μL of 1 × BB. The 50 μL of suspend were dropped onto the microscope glass slide and measured. A quantitative analysis of *S. typhimurium* was performed based on the measured peak area at 1628 cm^{-1} in the SERS spectrum.

2.7. Preparation of milk samples

Five milliliters of the milk sample was pretreated by centrifugation separation (10 °C, 7000 rpm) for 10 min, and the upper cream layer was removed. Subsequently, the supernatant was filtered through a 0.45-μm filtration membrane and diluted with ultrapure water at a 1:20 ratio. A series of known quantities of *S. typhimurium* were then added to the prepared samples for the experiments.

3. Results and discussion

3.1. Sensing strategy

A schematic illustration of the SERS-based aptasensor process for *S. typhimurium* determination is shown in Fig. 1. Au@Ag core/shell NPs were first synthesized as SERS-active substrates. Then, apt 1 bound with *S. typhimurium* was conjugated with Au@Ag core/shell NPs and utilized as capture probes. In the presence of the *S. typhimurium* target, Au@Ag-apt 1 recognized and bonded to *S. typhimurium*. After the subsequent addition of ROX-modified apt 2, Au@Ag-apt 1-target-apt 2-ROX sandwich-like complexes formed based on the high affinity and specificity of aptamer and *S. typhimurium*. As more *S. typhimurium* was added, the surface loading of bound ROX-apt 2 increased, resulting in increased SERS intensity. By monitoring the SERS signal increase along with changes in concentration, highly sensitive quantification of *S. typhimurium* could be realized.