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Ultrasensitive SERS detection of *Bacillus thuringiensis* special gene based on Au@Ag NRs and magnetic beads

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ABSTRCACT

Highly sensitive and selective detection of specific DNA sequences is of great importance in clinical diagnosis, environmental and food monitoring, but it still remains challenges to develop a facile method for real sample detection in aqueous solution. Here, a simple and recyclable surface enhanced Raman scattering (SERS) sensor was constructed for *Bacillus thuringiensis* (Bt) special gene fragment detection by Fe_3O_4 magnetic beads (MBs) and Au–Ag core-shell nanorods (Au@Ag NRs). A hairpin DNA with sulfhydryl and biotin was attached to Au@Ag NRs as indicator, and MBs with streptavidin (SA) were acted as the capture probe. On the basis of the biotin–SA specific interaction, target sequences were first hybridized with the hairpin DNA and exposed the biotin. Subsequently, the Au@Ag NRs were captured by the streptavidin modified MBs, which reduced the suspended NRs and led to the change of Raman intensity. Under the optimal conditions, the SERS intensity revealed a good linearity with Bt transgene fragment ranging from 0.1 pM to 1 nM with a detection limit of 0.14 pM (S/N=3). To demonstrate the specificity of the strategy, the single-base mismatch in DNA was discussed in the SERS assay. The results showed that the sensitivity and accuracy of the proposed method was acceptable in DNA detection, revealing a great potential in special gene detection.

1. Introduction

Bacillus thuringiensis (Bt), a specific DNA sequence, can produce insecticidal crystal protein, which has been widely used in genetically modified technology to achieve the goal of insect control (Höfte et al., 1989). Until now, transgenic crops with exogenous Bt gene are commercially available worldwide. However, increasing concerns have been aroused over the safety of genetically modified products due to the possible potential risks (Quist and Chapela, 2001). So, it is of great importance to develop facile and rapid methods for transgene detection in food. Recently, various methods such as polymerase chain reaction-based assays (Hernández et al., 2005), fluorescence emission spectroscopy (Ma et al., 2015a, 2015b; Su et al., 2014), electrochemical method (Cui et al., 2014; Rasheed and Sandhyarani, 2015) and many other techniques (Tian et al., 2014; Zhang et al., 2007) have been applied to the detection of specific DNA sequences. These analytic techniques can achieve high sensitivity and specificity, but usually require complex sample pretreatment or harsh reaction conditions and long amplification period. Due to the specific advantages such as good sensitivity, unique spectroscopic fingerprint, against photobleaching and noninvasive data collection, SERS has become a promising and significant technique in food analysis and bioassays (Guicheteau et al., 2008; Félix-Rivera et al., 2011; Li et al., 2015; Xu et al., 2015a, 2015b; Schlucker, 2014; Harper et al., 2013;). Moreover, since the first application of SERS label probe reported in 1994 (Vo-Dinh et al., 1994), the SERS based gene detection has attracted great research interest and become one of the most widely used spectroscopic analysis tools (Chen et al., 2014; Kang et al., 2010; Xu et al., 2015a, 2015b; Gao et al., 2012; Li et al., 2013).

Usually, SERS probes are consist of noble metal nanoparticles and special Raman molecules (Wang et al., 2013). Based on the relationship between electromagnetic effect and Raman intensity, nanoparticles with different geometric morphology have been designed as SERS substrate (Zhang et al., 2014a, 2014b) such as nanosphere (Song et al., 2014;Wang et al., 2014), nanorod (Zhang et al., 2013; Khlebtsov et al., 2013; Bai et al., 2014), nanostar (Zhang et al., 2015; Potara et al., 2013), nanoflower (Zhang et al., 2014a, 2014b; Senapati et al., 2011) and nanocluster (Lee et al., 2015). Wherein, the nanorods (NRs) possess greater activity than the corresponding spherical shaped nanoparticles due to the lightning-rod effect (Dong et al., 2014; Chen

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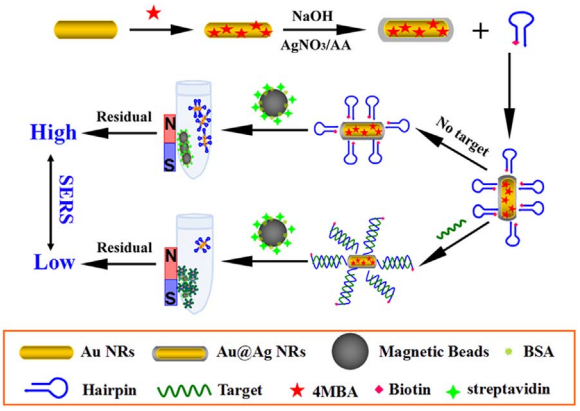
et al., 2013) and their plasmon resonance. It was reported that the deposition of Au on the surface of DNA-modified gold NPs (AuNPs) gave rise to enormous SERS enhancement (Lim et al., 2011). Moreover, it is known that in situ deposition of Ag in the presence of reporters can exhibit much stronger Raman signal than the corresponding Au nanostructures (Ma et al., 2014). Therefore, bimetallic core-shell nanostructures such as Au-Ag core-shell nanostructures could have more advantage than the single metal in physical and chemical properties. In fact, Au@Ag NRs have been extensively studied in many groups as SERS substrate (Dong et al., 2014; Chen et al., 2013; Ma et al., 2015a, 2015b). As for the SERS probe, embedded organic molecules in the gap of core-shell can produce intrametallic "hot spot" and prevent the interference from the surroundings, thus behaving amplified Raman signal and low background noise (Feng et al., 2012; Lee et al., 2014). Besides, Fe_3O_4 MBs possess the advantages such as ease of synthesis, facile surface modification, reliable stability and unique superparamagnetic properties, which have been widely applied in biological and medical fields (Wang et al., 2015; Cho et al., 2015; Tang et al., 2013). Especially, it is stable and efficient for MBs to disperse in the solution and capture targets (Liao et al., 2016). After the targets were captured, MBs can be easily separated and collected from the solution upon applying an external magnetic field. Thus, by combining bimetallic core-shell SERS substrate and MBs, it is encouraging to fabricate a multifunctional biosensors for biological assays and achieve the recycle use of substrate (Baniukevic et al., 2013; Zhao et al., 2015; Wen et al., 2013; Balzerova et al., 2014).

Herein, a SERS based strategy was designed for special Bt fragment sequence detection with the aid of Au@Ag NRs and magnetic beads (MBs) in aqueous solution (Scheme 1). Firstly, a hairpin DNA structure modified with sulfhydryl at the 3′-end and biotin at the 5′-end was attached to the Au@Ag NRs. Next, MBs modified with SA (MBs@SA) was utilized to capture Au@Ag NRs for convenient separation. In the absence of target, the stem-loop structure was closed and the biotin was masked, thus the biotin modified NRs cannot be captured by the MBs@SA. To the contrary, the loop would hybridize with the target and opened the hairpin so as to be captured by the SA, which reduced the suspended NRs and led to the change of Raman intensity. The SERS intensity behaved a good linearity and high sensitivity for Bt transgene detection in real sample. Hence, the SERS sensor is expected to be a useful analytical tool for detection of specific DNA sequences in clinical diagnosis, environmental and food monitoring.

2. Material and methods

2.1. Chemicals and materials

Cetyltrimethyl ammonium bromide (CTAB) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), ascorbic acid (AA), silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), hydrochloric acid (HCl), Magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), disodium phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), and diethylene glycol(DEG) were purchased from Sinopharm Chemical Reagent Co., Ltd. Polyacrylic acid (PAA, MW=~3000) and iron(III) chloride anhydrous ($FeCl_3$) were obtained from Aladdin Chemistry Co., Ltd. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 4-mercaptobenzoic acid (4-MBA) were obtained from Tokyo Chemical Industry Co., Ltd. SA and the oligonucleotides (Table S1) used in the work were purchased from Shanghai sangon Biotechnology Co., Ltd. Ultrapure water (≥18 MΩ, Milli-Q, Millipore) was used throughout the experiment.



Scheme 1. Schematic illustration of SERS detection of special DNA sequences.

2.2. Instrumentation

Ultraviolet-visible (UV–vis) absorption spectrum was obtained with a Nicolet Evolution 300 Ultraviolet-Visible spectrometer. The transmission electron microscope (TEM) images were acquired on a JEM-2100F transmission electron microscopy at an accelerating voltage of 200 kV. The surface potential were measured by dynamic light scatting (DLS) using a Malvern Zeta Sizer (Nano-ZS) system. Atomic force microscopy (AFM) images were taken using SPM9700 atomic force microscope, Shimadzu, Japan. Fourier Transform infrared (FT-IR) spectra was collected on an Avatar 330 ThermoFisher Nicolets spectrometer. All Raman spectra were recorded at room temperature using an inVia Raman spectrometer (Renishaw, UK) equipped with a confocal microscope (Leica, German). A He-Ne laser (633 nm) was used as the excitation light source. The spectrometer was calibrated by the band of a silicon wafer at 520 cm^{-1}. The SERS spectra were acquired with 10 s exposure and one time accumulation.

2.3. Synthesis and modification of Au@Ag NRs

To obtain Au@Ag NRs, Au NRs were prepared according to previously reported seed-mediated growth method with slightly modification (Nikoobakht and El-Sayed, 2003). Firstly, the seed solution was prepared and left still at 30 °C for about 2 h. Secondly, the growth solution was prepared and subsequently introduced with seed solution and stored overnight at 30 °C. To remove the excess chemical, the resulting solution was centrifuged at 11000 rpm for 10 min and the obtained precipitate was redispered with ultrapure water for further use. To further get SERS active probe, 4-MBA was served as Raman reporter molecular and incubated with Au NRs solution for at least 5 h. Following that, $AgNO_3$ was added into the mixture to acquire 4-MBA indicator embedded Au@Ag NRs. Herein, different concentration of $AgNO_3$ was discussed. The detailed procedures were elaborated in **Supplementary Information**.

2.4. Synthesis and functionalization of MBs

Magnetic beads (MBs) were prepared based on Yin's high-temperature hydrolysis method with some modification (Ge et al., 2007). Typically, NaOH (50 mmol) was dissolved in 20 mL DEG and the solution was heated at 120 °C for 1 h under nitrogen protection, then the NaOH/DEG stock solution was cooled and kept at 70 °C. Next, PAA (4 mmol) and $FeCl_3$ (0.4 mmol) were dissolved in DEG (17 mL) and the mixture was heated to 220 °C for 30 min with vigorous stirring in a nitrogen atmosphere to form a transparent light yellow solution. After that, the NaOH/DEG stock solution (1.75 mL) was injected into the above solution and the temperature dropped to about 210 °C. The resultant mixture solution was heated for another 1 h to obtain the magnetic beads. Finally, the above solution were washed with ultrapure water for at least three times.

To prepare the SA modified magnetic beads, EDC and NHS (1:1, 2 mg/mL) were added into I mL of the above carboxy-terminated MBs solution and incubated for 1 h. Then the mixture was washed with PBS