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Engineered "hot" core–shell nanostructures for patterned detection of chloramphenicol

Wenjing Yan [a,*], Longping Yang [a], Hong Zhuang [b], Haizhou Wu [a], Jianhao Zhang [a,*]

[a] National Center of Meat Quality & Safety Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[b] Quality and Safety Assessment Research Unit, Agricultural Research Service, USDA, Athens, GA 30605, USA

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ABSTRACT

In this study, we described a novel method for highly sensitive and specific detection of chloramphenicol (CAP) based on engineered "hot" Au core-Ag shell nanostructures (Au@Ag NSs). Cy5-labeled DNA aptamer was embedded between the Au and Ag layers as a signal generator and target-recognition element, to fabricate uniform Au@Ag NSs with unexpected strong and stable SERS signals. The presented CAP can specifically bind to the DNA aptamer by forming an aptamer-CAP conjugate, and cause greatly decreased SERS signals of Au@Ag NSs. By using this method, we were able to detect as low as 0.19 pg mL^{-1} of CAP with high selectivity, which is much lower than those previously reported biosensors. Compared with the other SERS sensors that attached a dye in the outer layer of nanoparticles, this method exhibits excellent sensitivity and has the potential to significantly improve stability and reproducibility of SERS-based detection techniques.

1. Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic secreted by the bacterium *Streptomyces venezuelae*. Because it can inhibit bacterial activities by preventing elongation of the peptide chains, CAP has been widely used in the field of animal breeding and clinical to prevent and treat a variety of bacterial infections (Cao et al., 2012; Feng et al., 2015; Malmasi et al., 2015). However, this drug is easy to accumulate in liver once it was absorbed into the body. Even after it is discharged into the environment, it cannot be degraded in a short period of time (Hanekamp and Bast, 2015; Li et al., 2013). In turn, the drug residues can get into the body through livestock products or environment and eventually causes aplastic anemia, bone marrow suppression and other adverse effects (Jain and Tripathi, 2015; Shukla et al., 2011). For these reasons, CAP has been strictly banned in China, USA, and Canada in all food producing animals. In the past decades, the content of the drug residues in animal source food have triggered a series of trade disputes between China and EU, resulting in about seven hundred million dollars losses in each year. Thereupon, it is of considerable significance to develop a specific and ultrasensitive approach for the detection of CAP in food.

Traditional methods for CAP detection, such as liquid chromatography mass spectrometer (LC–MS) (Tian et al., 2013), gas chromatography (GC) (Liu et al., 2014) and high performance liquid chromatography (HPLC) (Vosough and Esfahani, 2013), usually need professional technology and rich experience, and suffer from the expensive, time-consuming and complex analytical procedures. In recent years, metal nanoparticle-based methods have attracted the most attention in the field of rapid and highly sensitive detection, due to their unique optical and chemical properties (Saha et al., 2012). Various methods have been developed based on metal nanoparticles, such as colorimetric (F Gao et al., 2015; H Gao et al., 2015), fluorescence (Berlina et al., 2013), electrochemical (Feng et al., 2015) and surface-enhanced Raman scattering (F Gao et al., 2015; H Gao et al., 2015; Gao et al., 2013). Among these approaches, surface-enhanced Raman scattering (SERS), as a molecular fingerprint spectrum, is one of the most powerful techniques for ultrasensitive detection of specific analyte in complex matrices (Osberg et al., 2012; Shin et al., 2015). By designing the construction of substrate materials, SERS signal can be amplified to 10^6–10^{14} orders of magnitude depending on the particle sizes, shapes and gap widths, reaching single molecule detection (Cecchini et al., 2013; Lim et al., 2010). Most importantly, SERS provides a non-destructive approach for identification of molecular species by the unique fingerprint, which effectively avoids the interference of background signal and improves the specificity of detection. Based all of these features, SERS has been widely used in various areas, such as food safety, environment, bio-diagnosis, medicine, chemistry, etc. (Li et al., 2010; Zhang et al., 2013).

* Corresponding authors.
E-mail addresses: ywj1103@njau.edu.cn (W. Yan), nau_zjh@njau.edu.cn (J. Zhang).

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Substrate materials are of great importance for the fabrication of a SERS sensor, which significantly affects the stability, repeatability and enhancement of SERS signals (Hugall and Baumberg, 2015). So far, aggregated metal nanoparticles (NPs) is the most common substrate material, because the hot junction formed between aggregated NPs can strongly enhance SERS signals (Wang et al., 2013a, 2013b, 2013c). However, it is difficult to control the aggregation degree of NPs. The random distribution of hotspots and the poor repeatability of SERS signals remain challenging (Alvarez-Puebla and Liz-Marzan, 2010). Recently, some progresses have been made in the fabrication of self-assembled SERS-active nanostructures, such as dimers (Ma et al., 2013), trimers (Chen et al., 2010), pyramids (Xu et al., 2015a, 2015b), satellites (Gandra et al., 2012) and chains (Xu et al., 2015a, 2015b). Some of these discrete assemblies show strong SERS signals with high enhancement factor (EF) values range from 1.0×10^3 to 1.0×10^9, but still face challenges in the complex assembly process, low production yield, variability in particle distance and poor repeatability (Halas et al., 2011). Thus, developing a stable substrate material with high SERS enhancement is highly advantageous for ultrasensitive detection.

Gold core-silver shell nanostructures (Au@Ag NSs), made of an inner layer gold NPs encapsulated with silver materials, has been proven to be one of the most promising artificial SERS-active substrates (Lim et al., 2011). On the one hand, embedding Raman reporters insides between Au and Ag layers strongly enhance SERS compared to pure Au or Ag NPs by themselves, due to enhanced electric field formation between two closely metal layers (Wang et al., 2013a, 2013b, 2013c). On the other hand, a thin silver shell not only prevents the aggregation induced SERS enhancement, but also limits the diffusion of the Raman dye and the changes of hotspot regions (Shen et al., 2015). These factors are crucial for achieving a reliable, reproducible and ultrasensitive SERS nanoprobe. In the past decades, most attentions have been focused on the fabrication of SERS "tags" based on SERS-active Au@Ag NSs (Wang et al., 2013a, 2013b, 2013c). However, there are few studies on the utilization of the high SERS enhancement of Au@Ag NSs for direct detection of contaminants in food.

Here, we demonstrate that an Au@Ag NSs-based SERS active platform for CAP detection can be easily prepared by taking advantage of the high specific recognition between CAP and aptamer, and the strong SERS enhancement of core–shell nanostructures. Au NPs modified with Cy5-labeled ds DNA (aptamers inserted) were used as a seed to synthesize Au core-Ag shell nanostructures with strong SERS signal from Cy5 dye. The presented CAP can competitively bind with the aptamer, and cause the dissociation of aptamer from the surface of Au NPs, and further lead to a drastically decreased Raman signal intensity. Based on the relationship between the concentration of CAP and the SERS signals intensity, a high sensitive and selective sensing platform for CAP detection was achieved. The feasibility of the approach for real-world applications was also demonstrated by the detection of CAP in spiked milk samples.

2. Materials and methods

2.1. Materials and reagents

Chloroauric acid ($HAuCl_4$), sodium citrate, silver nitrate, ascorbic acid, polyvinylpyrrolidone; chloramphenicol (CAP), kanamycin (KAN), gentamicin (GEN), tetracycline (TC), thiamphenicol (TAP), streptomycin (SM) were purchased from Sigma-Aldrich (Shanghai, China). Thiolated DNA oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd, and were purified by polyacrylamide gel electrophoresis (PAGE). The DNA sequences used in this work are as follows:

DNA1: 5′ SH-TTT TAC CAC CGA CTC GCC-3′

DNA2 (aptamer): 5′-ACT TCA GTG AGT TGT CCC ACG GTC GGC GAG TCG GTG GTA G-Cy5 3′

2.2. Synthesis of Au NPs

Au NPs with a diameter of 10 nm were synthesized by reduction of $HAuCl_4$ using trisodium citrate combined with tannic acid. Briefly, 1 mL of 1% $HAuCl_4$ was added to 79 mL of deionized water. The solution was heated to 60 °C for 40 min (solution A). Four mL of 1% sodium citrate, 0.1 mL of 1% tannic acid, and 0.1 mL of 25 mM K_2CO_3 were added to 15.8 mL of deionized water. The solution was heated to 60 °C for 40 min (solution B). Then, solution B was quickly added to solution A under high speed stirring. The mixture was heated at 60 °C for 50 min. After the heat source was removed, it was cooled down to room temperature.

2.3. Fabrication of Au–DNA conjugates

DNA1 and DNA2 at the concentration of 2 μM were mixed in a 1:1 ratio in 10 mM PBS buffer. The solution was heated at 90 °C for 5 min and then slowly cooled to room temperature to obtain a ds DNA, it was stored at 4 °C before using. Five hundred μL of Au NP solution was centrifuged at 13,000 rpm for 10 min, the supernatant was removed, and the pellet was resuspended in 500 μL ds DNA solution.

The mixture of Au and ds DNA synthesized above was incubated for 30 min at room temperature and was salted by increasing the concentration of NaCl. Every 3 h, the salt concentration was increased by 0.05 M to reach the final concentration of NaCl at 0.3 M in 24 h. The Au–DNA conjugates were centrifuged (13,000 rpm, 10 min) to remove the free (unattached) ds DNA. The precipitate was re-suspended in 10 mM PBS buffer.

2.4. Synthesis of SERS-active Au@Ag NSs

Fifty μL of 5 fold concentrated Au–DNA conjugates were dispersed into a solution containing 200 μL of 10 mM PBS, 100 μL of 1% PVP and 50 μL of 0.1 M sodium ascorbate. Then, different amounts of 1 mM $AgNO_3$ solution (0 μL, 30 μL, 50 μL, 70 μL and 100 μL) were added to synthesis of Au@Ag NSs with different thickness of Ag shells.

2.5. Fabrication of SERS SENSOR for CAP detection

Au–DNA conjugates prepared in 2.3 were transferred to eight separated tubes (200 μL in each tube), and 5 μL of the CAP standard solution with different concentrations were added to make a final concentration of 0, 1, 5, 10, 50, 100, 500, and 1000 pg mL^{-1}. The mixtures were incubated for 40 min at room temperature with constant shaking and then was centrifuged at 1300 rpm for 10 min. The precipitates were resuspended in 10 mM PBS buffer and then coated with a Ag shell based on Section 2.4. The samples were characterized by LabRam-HR800 Micro-Raman spectrometer. An air-cooled He–Ne laser giving 514 nm excitation was used as the excitation source with an acquisition time of 20 s, the laser power used for SERS detection is ~10 mW. A standard curve was established based on the logarithmic relationship between CAP concentration and SERS signal intensity.