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Structure-selective hot-spot Raman enhancement for direct identification and detection of trace penicilloic acid allergen in penicillin

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ABSTRACT

Trace penicilloic acid allergen frequently leads to various fatal immune responses to many patients, but it is still a challenge to directly discriminate and detect its residue in penicillin by a chemosensing way. Here, we report that silver-coated gold nanoparticles (Au@Ag NPs) exhibit a structure-selective hot-spot Raman enhancement capability for direct identification and detection of trace penicilloic acid in penicillin. It has been demonstrated that penicilloic acid can very easily link Au@Ag NPs together by its two carboxyl groups, locating itself spontaneously at the interparticle of Au@Ag NPs to form strong Raman hot-spot. At the critical concentration inducing the nanoparticle aggregation, Raman-enhanced effect of penicilloic acid is ~60,000 folds higher than that of penicillin. In particular, the selective Raman enhancement to the two carboxyl groups makes the peak of carboxyl group at C_6 of penicilloic acid appear as a new Raman signal due to the opening of β-lactam ring of penicillin. The surface-enhanced Raman scattering (SERS) nanoparticle sensor reaches a sensitive limit lower than the prescribed 1.0‰ penicilloic acid residue in penicillin. The novel strategy to examine allergen is more rapid, convenient and inexpensive than the conventional separation-based assay methods.

1. Introduction

Penicillin has been one of the most widely used and highly-efficient antibacterial drugs for the clinical treatments of various infectious diseases, and saves a large number of lives of human as well as animals. However, allergic test is a necessary procedure before its use because the allergic responses for some patients are very serious and even fatal, for example, urticarial lesion, hypotension and anaphylactic shock (Mendelson et al., 1984; Pichichero, 2005; Sogn, 1984; Torres et al., 1999). It has now been clarified that penicilloic acid (PA) as the by-product in the synthesis of penicillin or the degradation product of penicillin in storage through the opening of β-lactam ring of penicillin is a major allergen causing the fatal immune responses by covalent conjugation with ε-amino groups of lysine residues of immunological proteins through penicilloyl groups (Batchelor et al., 1965, 1967; Blanca et al., 2001; Levine and Ovary, 1961; Smith and Marshall, 1971; Weltzien and Padovan, 1998). In general, the residual amount of PA less than 1.0% (w/w) in penicillin can significantly reduce the occurrence of allergic reactions. Therefore, the identification and measurement of trace PA residue in penicillin are important for the prevention of allergic reactions. Up to date, some detection methods for the screening of PA in penicillin have been reported, for example, colorimetric method (Pan, 1954), nuclear magnetic resonance (Degelaen et al., 1979), liquid chromatography–electrospray ionization mass spectrometry (Li et al., 2008) and enzyme-linked immunosorbent assay (Zhang et al., 2010). Due to the structural similarity to penicillin and the very trace amount in penicillin, the identification of PA usually requires the sophisticated sampling, beforehand separation/extraction and pre-concentration procedures in these above techniques, which are highly expensive or time-consuming and cause environmental problems. The method for the direct identification and detection of trace PA residue in penicillin has been a challenge.

With the integration of high sensitivity, unique spectroscopic fingerprints and nondestructive data acquisition, surface-enhanced Raman scattering (SERS) technique has become one of the most widely pursued spectroscopic tools for the identification and detection of chemical and biological species (Abbas et al., 2013; Qian and Nie, 2008; Smith, 2008). Theory of SERS is now largely established, in which most of the spectroscopic enhancement is attributed to the concentration of the electromagnetic (EM) optical fields near gold (Au)

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or silver (Ag) nanoparticles (Jeanmaire and Van Duyne, 1977). The amplification of Raman signals can lead to a 10^6–10^{15} enhancement depending on the strength of EM experienced by the molecules at the surface of various metal nanostructures (Nie and Emory, 1997; Pieczonka and Aroca, 2008). Recently, various Ag and Au nanoparticle colloids have been widely investigated to develop highly SERS-active substrates (Brus, 2008; Camden et al., 2008a; Li et al., 2013; Wu et al., 2012). In particular, the core–shell Au@Ag nanoparticles exhibit stronger Raman enhancement than pure Au or Ag NPs owing to the wide and strong plasmonic resonance absorption, and can be used as stand-alone-particle Raman amplifiers, as reported in our previous work (Liu et al., 2012). On the other hand, the highest Raman enhancement usually occurs at the interparticle "hot-spot" in which the electromagnetic optical fields are highly concentrated (Camden et al., 2008b; Pieczonka and Aroca, 2008; Qian and Nie, 2008). It is thus expected that the ultrahigh hot-spot enhancement of Au@Ag interparticles can provide a direct spectroscopic identification of trace analytes in a structure-like matrix substance.

Here, we report the direct identification and detection of trace PA residue from penicillin using a SERS nanoparticle sensor. The interparticle hot-spot of Au@Ag NPs can be induced by PA linkage to Au@Ag NPs through the surface complexing interaction of two carboxyl groups of PA. In the presence of a large amount of penicillin, the hot-spot can selectively enhance the Raman signals of PA, and result in the characteristic Raman fingerprint of PA different from that of penicillin. The rapid, in situ assay of trace PA residue in penicillin has been thus achieved with the excellent sensitivity, selectivity and good repeatability.

2. Experimental section

2.1. Reagents and materials

Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, 99.8%), chloroauric acid ($HAuCl_4 \cdot 4H_2O$, 99.9%), silver nitrate ($AgNO_3$, 99%), and ascorbic acid (99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Benzylpenicillin sodium was obtained from the North China Pharmaceutical Group Corporation (Shijiazhuang, China). All of these reagents were used without further treatment. Ultrapure water (18.2 MΩ cm) was produced using a Millipore water purification system (Milford, MA, USA).

2.2. Synthesis of Au@Ag NPs

The Au@Ag NPs were prepared using a two-step reduction method including the synthesis of Au cores and the subsequent growth of Ag shells, as described in our previous work (Du et al., 2013). The final concentration of Au@Ag NPs was estimated to be ~1.6 nM. See the details of synthesis in Supporting information.

2.3. Preparation and purification of PA

PA was prepared by alkaline hydrolysis of penicillin (Ghebre-Sellassie et al., 1984). Typically, 3.73 g of benzylpenicillin sodium was dissolved in 100 mL of 0.2 M NaOH at room temperature. The mixture was continuously stirred using a magnetic stirrer, and the pH remained greater than 12 in the reaction process. After 90 min, the pH was adjusted to 8.7 by the addition of 1.0 M HCl, and then the mixture was lyophilized. The resultant product was dissolved in anhydrous ethanol. PA crystallized from the ethanol solution at 4 °C after moderate acetonitrile was slowly added into above solution under rapid stirring. The characterization of mass spectroscopy (MS) gave *m/z*: 353.1 $[M+1]^+$ for the final product (the inset of Fig. S3B), which was completely consistent with the molecular weight of PA.

2.4. Theoretical calculations

The conformation search of PA molecule and the molecular vibrational bands of the investigated compounds were performed with a Gaussian 09 program package. See Supporting information for details.

2.5. Measurements of SERS of PA and penicillin

In brief, 0.5 M PA solution was prepared and diluted with ultrapure water to the predetermined concentrations. Then, 10 μL of the analyte solution was added into 90 μL of Au@Ag NPs colloids in a 1.5 mL centrifuge tube, followed by shaking for 1 min. The mixture was first sucked into a capillary glass tube, which was then fixed onto a glass slide. The Raman spectra were recorded using a 532-nm laser with 10 mW power and 10 × objective. The collecting time was 10 s with 5 rounds of accumulations and the pinhole aperture was 25 μm. Penicillin was also tested using the same method.

2.6. SERS detection of PA in spiked penicillin

Briefly, PA with different amounts was first spiked into 1 g of penicillin, and then the mixture was dissolved into 10 mL ultrapure water. After the aqueous solution was diluted 10 times, 10 μL of spiked penicillin solution was added into 90 μL of Au@Ag NPs colloids. The Raman spectra were directly recorded from a glass capillary tube loaded with the mixing solution using the 532-nm laser with 10 mW power and 10 × objective. The collecting time was 30 s with 5 rounds of accumulations and the pinhole aperture was 25 μm.

Accuracy of the method was determined by a standard addition technique. Known amounts of reference standard PA in a range of low, medium and high concentrations were added to preanalyzed sample of commercial penicillin and analyzed under the above measuring conditions. The experiments were replicated five times for each concentration and the accuracy was calculated as the % of analyte recovered.

2.7. Characterization and instruments

The morphologies and structures of nanoparticles were examined by a JEM-1010 transmission electron microscope (Tokyo, Japan). The hydrodynamic sizes of nanoparticles were determined using a Malvern Zetasizer Nano-ZS90 particle size analyzer (Malvern Instrument, UK). UV–vis absorption spectra were obtained by a UV-2501 spectrometer (Tokyo, Japan). Raman measurements were conducted with a Thermo Fisher DXR Raman Microscope equipped with a CCD detector in backscattered configuration using a 10 × objective (Madison, USA). The structure identification for target analyte was performed using an Agilent 6410B Triple Quad LC–ESI–MS/MS (MassHunter Data Acquisition, Qualitation and Quantitation software, USA).

3. Results and discussion

3.1. Characterizations of Au@Ag NPs

The core–shell Au@Ag NPs were prepared by seed growth through a consecutive two-step reduction process (see Supporting information) (Du et al., 2013). 30-nm Au cores were first synthesized by the chemical reduction of chloroauric acid with sodium citrate, and used as seeds for the formation of Ag nanoshells. Subsequently, silver nitrate was added into the above seed colloids and reduced with ascorbic acid under vigorous stirring, leading to