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Kanamycin detection based on the catalytic ability enhancement of gold nanoparticles

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ABSTRACT

In this paper, we demonstrated that kanamycin could enhance the peroxidase-like activity of citrate-capped gold nanoparticles (AuNPs) through two steps: the attachment of kanamycin onto AuNPs through $-NH_2$ (on kanamycin) and −COOH (on AuNPs) interactions; and the specifically interaction between glucoside on kanamycin and AuNPs which changes the surface property of AuNPs, and produced •OH radicals and Au^{3+} in the solution, and catalyzed the chromogenic reactions between 3, 3′, 5, 5′-tetramethylbenzidine (TMB) and H_2O_2. Based on this principle, a novel method for kanamycin detection has been developed. This method exhibited high sensitivity and selectivity, as low as 0.1 nM kanamycin could be detected with a linear range from 0.1 nM to 20 nM and 20nM to 300nM, respectively. This method was also successfully applied for the detection of kanamycin content in milk and meat samples.

1. Introduction

In recent years, on account of their unique optical, electronic, magnetic and biocompatibility characters, metal or alloy nanomaterials have aroused people's much attention, and are widely applied for biosensing (Bhattacharya and Mukherjee, 2008; Kumar et al., 2015; Bhattacharjee et al., 2016; Dutta et al., 2016), separation (Chen et al., 2016; Yildiz, 2016), imaging (McCarthy and Weissleder, 2008; Smith et al., 2008), drug delivery (Ghosh et al., 2008) and diagnostic application (Kim et al., 2016). Among these materials, the study and application of gold nanoparticles (AuNPs) has been of great interest (Kim et al., 2016; Nie et al., 2014). Its catalytic ability, namely peroxidase-mimetic property was also utilized by people in the biosensing assay (Wang et al., 2011b; Long et al., 2011; Zhu et al., 2013; Tao et al., 2013). However, the exact mechanism or process of these small Au species involved catalytic reactions are ambiguous or not consistent. The detected substances are also limited (*e.g.*, mainly include Hg^{2+} that can form alloy with Au or molecules that can produce H_2O_2 in the presence of specific oxidase). Thus, it is urgent to explore the exact mechanism of AuNPs act as enzyme-like nanomaterials and it is desirable to develop a simple and inexpensive method for sensitively detecting more biological species, *e.g.*, harmful substances in the agricultural and food products that threaten people's health.

Antibiotics are widely used in the process of food production and livestock husbandry (Strachan and Davies, 2016; Tasho and Cho, 2016). However, due to its overuse, kanamycin residues in animal-derived food (*e.g.*, milk, meat and eggs) may lead to different degree of damage, including ototoxicity, nephrotoxicity and allergic reactions, et al. (Groschel et al., 2016; Liu et al., 2016). The European Union (EU) has established maximum residue limits (MRL) for kanamycin in edible tissues and milk: 200 nM for meat and 300 nM for milk (Song et al., 2011; Xing et al., 2015). So far, lots of analytical methods have been reported for detecting kanamycin, such as gas chromatograph (GC) (Preu et al., 1998), high performance liquid chromatography (HPLC) (Manyanga et al., 2010; Patel et al., 2015), liquid chromatography-mass spectrometry (LC-MS) (Oertel et al., 2004) and enzyme-linked immunosorbent assay (ELISA) (Chen et al., 2008; He et al., 2016; et al). Although these methods can efficiently detect kanamycin, there are still some hindrances, such as time-consuming, high cost, tedious and difficult operation, or are susceptible to interferences. Therefore, it is desirable and urgent to develop facile, cost-efficient and rapid assays for the detection of kanamycin with favorable sensitivity and selectivity.

Here, we found that kanamycin has the ability to enhance the catalytic ability of AuNPs by increasing the amounts of Au^{3+} and •OH in the solution, which improve the oxidation degree of TMB in the presence of H_2O_2 and produced blue-colored species. This proposed method shows high selectivity and sensitivity, and can be used to detect kanamycin in milk, pork and chicken samples with satisfactory results.

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2. Experimental section

2.1. Chemical and materials

$HAuCl_4 \cdot 4H_2O$ was purchased from Sigma-Aldrich (St. Louis, MO). Antibiotics (kanamycin sulfate, streptomycin sulfate, neomycin sulfate, sisomicin sulfate, tobramycin sulfate, ribostamycin sulfate and spectinomycin sulfate) and TMB were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Aptamer with the following sequence: 5′-TGGGG GTTGA GGCTA AGCCG A-3′ was chosen according to the previous report (Wang et al., 2016) and obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China)·H_2O_2, NaCl, trisodium citrate dihydrate, acetic acid, cystamine, Tris-hydroxymethyl aminomethane (Tris), dimethyl pyridine N-oxide (DMPO) and HCl were acquired from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Glucose, maltose, lactose, fructose, sucrose, sodium gluconate, gluconolactone, N-methyl-D-glucamine, β-D-glucose pentaacetate, β-D-glucosamine pentaacetate, N-acetyl-D-glucosamine, D-glucosamine hydrochloride were acquired from Shanghai Titan Scientific Co., Ltd (Shanghai, China). The milk, pork and chicken samples were acquired from a local supermarket. All other chemicals were of analytical grade and used without further purification. Double distilled water (dd H_2O) was used throughout the experiment.

2.2. Apparatus

The UV–visible (UV–vis) absorption spectra were recorded on a Tecan Infinite 200 PRO microplate reader (Mannedorf, Swiss). The Fourier transform infrared (FTIR) spectra (4000–650 cm^{-1}) were recorded with a Nicolet IS10 FTIR spectrometer (Thermo Fisher Co., Ltd., USA). pH values of the solutions were adjusted using a Leici PHS-3E pH meter (INESA Scientific Instrument Co., Ltd., China). The centrifugation process was operated using a CF-10 centrifuge (WiseSpin Co., Ltd., Korea). All measurements were carried out at room temperature otherwise mentioned specially.

2.3. Synthesis of AuNPs

13 nm AuNPs were prepared by reducing $HAuCl_4$ with trisodium citrate according to the previous report (Wang et al., 2011a, 2010b). The concentration of AuNPs was determined by UV–vis spectroscopy as 6.7 nM (the molar extinction coefficient is 2.7×10^8 M^{-1} cm^{-1} at 518 nm (Demers et al., 2000).

2.4. Detection of kanamycin in aqueous solutions

10 μL different concentrations of kanamycin, 50 μL Tris-HCl buffer (25 mM), 120 μL ddH_2O and 20 μL AuNPs were added into 96 wells plate successively. After mixed thoroughly and reacted for 10 min, a mixture of 50 μL Tris-HCl (25 mM), 25 μL TMB (6.7 mM) and 25 μL H_2O_2 (2 M) were added into the wells, after reacted for 45 min, the UV–vis absorption spectra of the final solutions were recorded from 500 to 800 nM.

2.5. X-ray photoelectron spectroscopy (XPS) measurement

XPS spectra were collected using a Thermo ESCALAB250 X-ray photoelectron spectroscope (Thermo, UK). AuNPs solutions in the absence and presence of kanamycin were dropped on a Si substrate and oven-dried before measurement.

2.6. Electron paramagnetic resonance (EPR) measurement

EPR signal was measured by a Bruker ESP 300E spectrometer (Bruker, Rheinstetten, Germany) with microwave bridge (receiver gain, 1×10^5; modulation amplitude, 2 Gauss; microwave power, 10 mW; modulation frequency, 100 kHz). A sample containing 0.1 M DMPO was transferred to a quartz capillary tube and placed in the EPR cavity. Under the UV-irradiation at 355 nm, EPR signal was detected using DMPO as the spin trap.

2.7. Real samples pretreatment and detection

Different amounts of kanamycin were added into 10 mL milk samples, then 20% (v/v) acetic acid was added gradually until the pH of the solution reached 4.5, after the solutions were centrifuged at 13,200 r/min for 15 min, the supernatant was pipetted and filtrated using a 0.22 μm membrane, the filtrate was collected. The fat and other organs were removed from 5g pork or chicken, and then the meats were shattered sufficiently using surgical scissors. After adding 8 mL Tris-HCl buffer (pH 7.5, 25 mM) and mixed thoroughly for 5 min, the mixtures were centrifuged at 3000 r/min for 20 min, then different amounts of kanamycin were added into the supernatant. The solutions acquired by pretreating milk, pork and chicken were detected using our developed method.

3. Results and discussions

3.1. AuNPs as peroxidases for detecting kanamycin

According to previous reports, TMB can be oxidized by H_2O_2 and produce a blue color product with a significant maximum absorbance at 650 nm (Wang et al., 2015), as shown in Fig. S1. After adding AuNPs into the TMB-H_2O_2 solution, the reaction of TMB and H_2O_2 can be catalyzed by citrate-capped AuNPs, with further addition of kanamycin, the catalytic activity of AuNPs was further improved, and the color of the mixture was also changed to deep blue, enabling the colorimetric determine the concentration of kanamycin in solutions.

3.2. Optimization of detecting conditions

In order to select the optimal experimental conditions for detecting kanamycin, the pH value of the solution, the amounts of TMB and H_2O_2 and the reaction time were carefully studied. As shown in Fig. 1A and S2, while varying the pH of the solution from 4 to 7.5, the color intensity and the absorbance of the mixture increased no matter there is kanamycin or not, the optical densities (OD) at maximum absorbance wavelength of 650 nm (OD_{650}) were also increased consequently. ΔOD_{650} ($OD_{kanamycin}-OD_0$) was used as a criterion to optimize the detection conditions, where the $OD_{kanamycin}$ and OD_0 is the OD_{650} of the solutions in the presence and absence of kanamycin, respectively. The ΔOD_{650} was increased until pH value was 7.5. this may because that TMB-H_2O_2 chromogenic reaction would be inhibited at lower pH (Li et al., 2009); on the other hand, higher pH value may facilitate the interaction between kanamycin and AuNPs by the electrostatic interactions. Nevertheless, under much higher pH condition, H_2O_2 becomes unstable and tends to decompose into H_2O and O_2 (Wang et al., 2010c). Therefore, pH 7.5 was chosen in the following experiment.

The amounts of TMB and H_2O_2 are of great importance in the TMB-H_2O_2 chromogenic system, as shown in Fig. 1B and S3, with increasing the amounts of TMB and H_2O_2 simultaneously, the color intensity and the absorbance of the solution is increased consequently, the OD_{650} of the solution was also increased significantly. When the final concentrations of TMB and H_2O_2 are 0.56 mM and 0.17 M (*i.e.*, 25 μL TMB and 25 μL H_2O_2 in the solutions), the ΔOD_{650} was highest, if the concentrations still increased, the background signal in the absence of kanamycin is too high, which results in the ΔOD_{650} decreased, consequently (Li et al., 2009). Therefore, 0.56 mM TMB and 0.17 M H_2O_2 were used in the following experiment. The reaction time after adding TMB and H_2O_2 into the solution was also investigated, as shown in Fig. 1C, and the ΔOD_{650} increased with increasing the reaction time from 0 to 45 min, and kept constant after 45 min. So,