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Ultrasensitive SERS aptasensor for the detection of oxytetracycline based on a gold-enhanced nano-assembly

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ABSTRACT

This paper investigated a new detection method of oxytetracycline (OTC) in aquatic products with ultrasensitive detection limit. The method was constructed on the basis of raman hot spot between gold nanoparticles (AuNPs) (13 nm and 80 nm diameter respectively) linked by an DNA sequence. The DNA sequence combined with the OTC aptamer including its complementary sequence as well as a stem-loop structure. The raman signal molecule (4-MBA) was modified at the surface of 13 nm AuNPs. After the exposure of OTC, the aptamer sequence was preferentially combined with OTC and partially dehybridized with its complementary sequence which led the 13 nm AuNPs to get more closer to the 80 nm AuNPs. The raman intensity was thus increased for the more enhanced hot spot generated. Under the optimal experimental conditions, the SERS signal was positively related to the OTC concentration with a wide working range of 4.60×10^{-2}–4.60×10^{2} fg/mL and the limit of detection (LOD) was as low as 4.35×10^{-3} fg/mL. The recovery rates of fishmeal ranged from 91.29–110.98%. The specificity of this method was further examined, and the results showed that the AuNPs based aptasensor was highly selective. This developed ultrasensitive aptamer-based SERS detection platform suggested that it may be a promising strategy for a variety of sensing applications.

1. Introduction

Oxytetracycline (OTC) is a member of the broad-spectrum tetracycline (TC) group of antibiotics [1] containing four condensed aromatic rings [2]. TCs is widely used as antimicrobial agent or growth enhancer in animal feeding industry. However, drug abuse has led to OTC residues in animal foods emerging resistance in the human body [3], and poses serious risk to human health. In view of this fact, America, China and other country have kept tight control over the acceptable OTC level for different kinds of food.

Until now, various analytical methods have been established for the determination of OTC, including high-performance thin-layer chromatography (HPTLC) [4], liquid chromatography-mass spectrometry (LC/MS) [5], high performance liquid chromatography (HPLC) [6], gas chromatography-mass spectrometry [7], microbiological methods [8], fluorescence spectroscopy [9] and UV spectroscopy [10], enzyme-linked immunoassay [11] (ELISA), electrochemical [12] and so on. HPTLC is easy to operate and the result is visible, but not very sensitive. LC/MS, HPLC and gas chromatography-mass spectrometry require complicated instruments and operation and are time-consuming, although these methods provide accurate detection. Microbiological methods can provide reliable results on the basis of long time. ELISA has high specificity, but it is time-consuming(multiple incubations and washing steps) with high background absorption and susceptible to being influenced by sample matrix [13]. Fluorescence spectroscopy, UV spectroscopy and electrochemical method could provide simultaneous and accurate detection, simultaneously require sample pre-treatment. That is to say, simple, efficient, sensitive and specific method for the detection of OTC residues in food products is eagerly demanded.

Aptamers, first reported in 1990, are artificial short single-stranded oligonucleotides of DNA or RNA selected by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [14,15]. Comparing to traditional immunological and chemical recognition molecules, aptamers have better target versatility, stronger affinity, higher specificity, better stability because of their temperature tolerance and other physical conditions. Besides, aptamers are taking advantages in small molecular weights, nontoxicity, non-immunogenicity, and good osmosis in tissue. So far, aptamers have been widely used in analytical chemistry, biochemistry, food and other fields, which provides a new

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and efficient detection strategy for basic research, drug analysis, medical inspection and food safety [15–23]. Aptamers-based biosensor (aptasensor) has emerged as a powerful tool which can meet the requirements of simplicity, sensitivity and specificity for the detection of diverse substances at trace levels [23].

Among the existing sensing technologies, surface-enhanced Raman scattering (SERS), as a molecular fingerprint spectrum, is one of the promising technologies as it exhibits a unique blend of advantages, such as ultrasensitive [24], non-invasive probing, compatibility with aqueous solution, minimal sample preparation, and label-free monitoring for specific analyte in complex matrices [25]. Giving all of these features, SERS has been widely used in various areas, such as food safety, environment, bio-diagnosis, medicine, chemistry, etc. [26–29]. Aptasensor has been used in conjunction with SERS as SERS-active substrate. Substrate materials are of great importance for the fabrication of a SERS sensor, which significantly affects the stability, repeatability and enhancement of SERS signals [30]. SERS phenomenon was commonly observed from some nanometal substrate such as Ag, Au, Cu colloids that absorbed special molecules, and some nanoparticle assemblies such as dimers, tetrahedrons, satellites, chains helices, and nanocrystals [31], which caused raman scattering signal to greatly enhance compared with normal raman signal [32].

Herein, a nano-biosensor based on gold nanoparticles (AuNPs) with stem-loop DNA modification as SERS-active substrate for the detection of fg/mL level of OTC in food matrices was developed. The 13 nm AuNP was bonded exclusively to the 80 nm AuNP utilizing a 56 mer single-stranded stem-loop DNA (ssDNA) sequence containing the aptamer of OTC. The 13 nm AuNP was subsequently functionalized with the raman reporter molecule, 4-mercaptobenzoic acid (4-MBA). The SERS hot spot formed between the 80 nm AuNPs and the 13 nm AuNPs. OTC caused partial dehybridization of the DNA sequence allowing the 13 nm AuNPs to get closer to the 80 nm AuNPs. Thereby the dehybridization increased the electromagnetic field in the SERS hot spot existing in the gap between the 80 nm AuNPs and the 13 nm AuNPs. The SERS signal was positively related to the OTC concentration. The developed nano-biosensor was ultrasensitive, without much sample pretreatment, and can provide reliable detection of fg/mL level of OTC in food systems.

2. Experimental section

2.1. Materials

6-mercapto-1-hexanol (MCH, ≥97%), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP, ≥98.0%), avidin and chloramphenicol (≥99.9%) were obtained from Sigma-Aldrich (Shanghai, China). Sodium chloride (NaCl), chloroauric acid, sodium citrate, citric acid, L-ascorbic acid and 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Thiamphenicol, florfenicol, tetracycline hydrochloride and polyethyleneimine were purchased from Tokyo Chemical Industry Co. Ltd. OTC and 4-MBA were purchased from Aladdin Industrial Inc (Shanghai, China). Kanamycin sulfate and ampicillin sodium salt were purchased from Amresco (U.S.A). If not stated otherwise, water used during the procedure was from a Milli-Q device (18.2 MΩ, Millipore, Molsheim, France). DNA oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd (Shanghai, P.R. China) and suspended in water to a final concentration of 1 μM. The DNA sequence combined with the OTC aptamer [1] including its complementary sequence as well as a stem-loop structure. The predicted structure of this sequence is shown in Fig. S2. The positions for modification were marked respectively. Thiol group was modified between T and C on the loop part of the stem-loop DNA. The 5′ end of the DNA was used to modify with biotin. The DNA sequences [33,34] were as follows:

5′-biotin-ACCGCACCACCGTCATGAGTGCGAACTTACGCACT CATGACGGTGGTGCGGTGGTG -3′.

2.2. Preparation of gold nanoparticles (AuNPs)

The smaller AuNPs (13 nm) and the bigger AuNPs (80 nm) were synthesized according to the protocol of Christoph Ziegler [35]. The smaller AuNPs (13 nm) were prepared by the standard citrate reduction method. Briefly, 2.5 mL of 4.86 mM $HAuCl_4$ solution added to round-bottomed flask containing 50 mL of water was heated to boiling and then 2 mL of 34 mM sodium-citrate solution (containing 2.6 mM citric acid) was infused quickly under vigorous stirring. The solution was kept boiling for 5 min and was then allowed to cool down naturally. The preparation of the 80 nm AuNPs was synthesized based on the seed solutions via two growth steps. In the first step, 3 mL of seed solution and 17 mL of water were added to a three-necked flask with a stirrer. A 10 mL aliquot of 1 mM $HAuCl_4$ solution and 10 mL of the reducing solution (0.5 mL of 34 mM trisodium citrate, 1.75 mL of 56.78 mM ascorbic acid stock solution and 7.75 mL water) were successively added to the three-necked flask at room temperature via injection pump at a slow speed under vigorous stirring. After the addition was completed, the mixture was allowed to keep stirring for 20 min and then brought to boiling for about 30 min. Finally the solution was allowed to cool down. 4.5 mL of the resulting sol was used in the second step with again 10 mL of 1 mM $HAuCl_4$ solution, 0.5 mL of ascorbic acid and 1.75 mL of trisodium citrate solution. Gold sols with different particle diameters would used to test UV–vis spectroscopy and TEM.

2.3. Fabrication of SERS-active sensor for OTC detection

AuNPs with aptamers was assembled according to the method of Nam Hoon Kim [33]. Firstly, stem-loop DNA (1 mL of 1 μM) in aqueous solution was annealed for 30 min in a glass bottle at 65 °C water bath. Secondly, put the DNA solution at the 55 °C water bath for minutes. And then the DNA solution was suspended in the upper edge of the water bath so that it was slowly cooled to room temperature prevent dimerization of the DNA. 10 μL of 1 mM freshly prepared TCEP was added to the glass bottle to activate the thiol-modified DNA. The DNA solution was allowed to stand at room temperature for 1 h. The 80 nm AuNPs solution was added to the glass bottle and then the glass bottle was placed at 37 °C oscillator with gentle shaking for overnight, followed by three washings by centrifugation at 3500 rpm for 15 min with buffer (NaCl 5 mM, Tris 5 mM). The supernatant and sediment were collected for UV detection. Then the 80 nm AuNPs was passivated with 0.1 mL of 0.1 mM aqueous 6-mercapto-1-hexanol (MCH) solution to form passivation layer. After 1 h, the mixture was washed one time with buffer (NaCl 5 mM, Tris 5 mM) above method. An aqueous solution of avidin (1 mL of 7.35 nM) was added to the glass bottle at 37 °C with gentle shaking for overnight. After incubation, the substrates solution was washed three times with PBS buffer and once with distilled water, using the above method. The supernatant and sediment were once again collected for UV detection. The glass bottle after being added 1 mL of the 13 nm AuNPs solution to was placed in shocker at ambient temperature with gentle shaking for 12 h. After incubation, the solution was washed three times with distilled water. The supernatant and sediment were once again collected for UV detection. Then 1 mL of 1 mM 4-MBA methanolic solution was added to the glass bottle at 37 °C oscillator with gentle shaking for 12 h. After incubation, the solution was washed with distilled water and buffer solution (NaCl 5 mM, Tris 5 mM), and stored in buffer (NaCl 150 mM, Tris 5 mM) at 4 °C. The supernatant was once again collected for UV detection.

2.4. SERS detection of OTC

The sample was mapped with the raman band of 4-MBA at 1592 cm^{-1} with a spectral resolution of ~2 cm^{-1}. The collecting time was 15 s with 1 rounds of accumulations. SERS spectral collection