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Label-free aptamer-based sensor for specific detection of malathion residues by surface-enhanced Raman scattering

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ABSTRACT

A novel label-free aptamer surface-enhanced Raman scattering (SERS) sensor for trace malathion residue detection was proposed. In this process, the binding of malathion molecule with aptamer is identified directly. The silver nanoparticles modified with positively charged spermine served as enhancing and capture reagents for the negatively charged aptamer. Then, the silver nanoparticles modified by aptamer were used to specifically capture the malathion. The SERS background spectra of spermine, aptamer, and malathion were recorded and distinguished with the spectrum of malathion–aptamer. To enhance the characteristic peak signal of malathion captured by the aptamer, the aggregate reagents (NaCl, KCl, $MgCl_2$) were compared and selected. The selectivity of this method was verified in the mixed-pesticide standard solution, which included malathion, phosmet, chlorpyrifos-methyl, and fethion. Results show that malathion can be specifically identified when the mixed-pesticide interferences existed. The standard curve was established, presenting a good linear range of 5×10^{-7} to 1×10^{-5} mol·L^{-1}. The spiked experiments for tap water show good recoveries from 87.4% to 110.5% with a relative standard deviation of less than 4.22%. Therefore, the proposed label-free aptamer SERS sensor is convenient, specifically detects trace malathion residues, and can be applied for qualitative and quantitative analysis of other pesticides.

1. Introduction

Pesticide residues, which mainly include organophosphorus, organochlorine, pyrethroid and carbamic ester, have similar molecular structures, which make them difficult to separate and specifically detected. Nowadays, gas chromatography [1], high-performance liquid chromatography [2], liquid chromatography-mass spectrometry [3,4], and gas chromatography–mass spectrometry [5] are the main standard detection methods for pesticide residues. These methods possess the advantages of high sensitivity and good accuracy. However, they usually need complex pretreatment of samples, which is a time consuming process. Also, expensive laboratory instruments are inconvenient for use in on-site rapid detection and batch testing. Enzyme inhibition methods, including enzyme-linked immunosorbent assay (ELISA) [6] and biosensors [7], are developing quickly in the field of rapid detection for pesticide residues. Enzyme inhibition methods are mainly based on acetylcholinesterase inhibition, which means that the activity of acetylcholinesterase is indirectly related with the concentration of pesticides. However, enzyme inhibition methods present an average specificity because almost all pesticides have inhibition effects on acetylcholinesterase. A problem with other methods is that ELISA and biosensor with antibodies [8,9] are expensive, although they provide good specificity because of the antibody-antigen specific binding. Therefore, a rapid, specific and economical detection method is needed for pesticide residues.

Recently, molecularly imprinted polymer [10,11] or aptamer [12] was reported to replace antibody in specific detection. Nucleic acid aptamers are oligonucleotide fragments that are screened by in vitro screening techniques, and they specifically bind to proteins or other small molecules. It has shown a strong advantage in the rapid detection of small molecules in alternative antibodies. It has also been used for different targets [13] including metal ions, small organic molecules, peptides and proteins and even whole cells or microorganisms, combined with different technologies [14,15] including colorimetric, fluorescence, electrochemical, and Raman spectroscopy. Sassolas et al. [16] reported that the aptamer biosensor for pesticides will replace traditional quick pesticide detection methods. However, the screening of nucleic acid aptamers for pesticides has rarely been reported. Nowadays, reports have focused on substances such as acetaminophen [17], atrazine [18], and malathion [19–21]. Other reports have also been conducted on one aptamer [22] that can simultaneously detect iprobenfos and edifenphos and another aptamer [23] that can simultaneously detect phorate, profenofos, isocarbophos, and omethoate. By contrast, pesticide detection methods based on colorimetric, electrochemical impendence and surface-enhanced Raman scattering(SERS) usually

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need modified molecular indicators at the end of 5′ or 3′ on the aptamer molecules, such as electro mediator $K_3[FeCN_6]$, fluorescence mediator rhodamine B, or chemiluminescent reagents lumino. For SERS, Raman indicators, including ROX, rhodamine 6G, HEX, FAM, TET, Cy3, Cy5, TAMRA are always introduced [24]. The concentration of pesticides was identified by SERS signals that come from these indicators. However, all the above indicators are not very specific because the binding information between the aptamer and the other interference molecules cannot be transferred directly.

The use of label-free aptamer could improve the selectivity of the SERS detection method, although it has rarely been reported [25]. The binding detail between the label-free aptamer with malathion was obtained by identifying the difference of characteristic peaks of the probe and the target. In this paper, the characteristic peaks of label-free aptamer reacted with malathion were identified. Pesticides interferences molecules such as phosmet, chlorpyrifos-methyl, and fethion were also analyzed. The good specificity and recoveries showed that this SERS detection method could be used to analyze malathion and can be further introduced in the detection of other pesticides.

2. Experimental

2.1. Instruments and Reagents

Normal Raman and SERS spectra were recorded using LabRAM HR UV 800 Laser Micro Raman Spectrometer (JOBIN YVON, France) equipped with a CCD detector and coupled to an Olympus microscope (U-5RE-2) with a ×50 objective lens. A 632.81 nm laser excitation device (He–Ne laser) was used to irradiate samples and the laser power was set to ~1.5 mW. UV–visible absorbance spectra were recorded by a CARY100 UV–Vis spectrometer (Varian, USA). Transmission electron microscopy (TEM, Tecnai G2 F30 S-Twin, Philips-FEI, Holland) was used to analyze the shapes and sizes of Ag colloids.

Spermine(98% purity), $AgNO_3$, trisodium citrate, malathion, phosmet, chlorpyrifos-methyl and fenthion were purchased from Aladdin (Shanghai, China). Malathion aptamer was modified by SH functional group.

5′-SH-ATCCGTCACACCTGCTCTTATACACAATTGTTTTTCTCTTAACTTC TTGACTGCTGGTGTTGGCTCCCGTAT-3′ was purchased from Sangon Biotech (Shanghai) Co., Ltd. Phosphate-buffered solution (PBS, 0.1 $mol \cdot L^{-1}$, pH 7.4) was prepared by NaH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$. All the other chemical reagents, including $NaH_2PO_4 \cdot 2H_2O$, $Na_2HPO_4 \cdot 12H_2O$, Na_2SO_4, NaCl, and HCl were all of analytical grade and used without further purification. All the solutions were prepared with ultrapure water (18.3 MΩ·cm, Human, Korea).

2.2. Ag Colloids Preparation

Ag colloids were prepared by $AgNO_3$ and trisodium citrate based on the method proposed by Lee and Meisel [26] with a slight modification. First, 100 mL 10^{-3} $mol \cdot L^{-1}$ $AgNO_3$ was heated to boiling in a three-necked flask under stirring. Then, 5 mL 1% trisodium citrate solution was added drop wise. Then, the solution was kept boiling for 1 h. The color of the solution changed from colorless to brownish yellow, then to silver gray. The obtained silver gray colloids were cooled down slowly at the condition of stirring and room temperature. All glass vessels were dipped and cleaned by using aqua regia solution ($HCl:HNO_3 = 3:1$, with caution) and ultrapure water before experiments. Trisodium citrate served not only as a reducing reagent, but also as a stable colloid reagent. The proper proportion of $AgNO_3$ and trisodium citrate avoids the aggregation of colloids because the diffuse double layer structure was formed to maintain the stability of colloids. Finally, the prepared colloids were stored in a brown wide-mouthed bottle at 4 °C. Before the SERS test, the colloids were centrifuged at 10,000 $r \cdot min^{-1}$ for 5 min. Then, the supernatant was poured out. Ultrapure water was poured into the remaining solution and the mixture was ultrasonically dispersed for 5 min. Then, the concentrated metal colloids were obtained after centrifugation for two times.

2.3. AgNPs@Sp, Aptamer, Aptamer-malathion, and Pesticide Standard Solutions Preparation

The prepared Ag colloids were first mixed with an equal volume of 10^{-5} $mol \cdot L^{-1}$ spermine functioning as a SERS enhanced reagent and named AgNPs@Sp. Afterwards, the purchased malathion aptamer was diluted to 10^{-6} $mol \cdot L^{-1}$ by using a pH 7.4 PBS buffer solution. A 10^{-6} $mol \cdot L^{-1}$ malathion aptamer solution was mixed with an equal volume of malathion that has different concentrations and left the mixture to achieve equilibrate for 20 min; this combination was named aptamer-malathion. Malathion standard solutions of 5×10^{-7}, 1×10^{-6}, 2.5×10^{-6}, 5×10^{-6}, 7.5×10^{-6}, and 1×10^{-5} $mol \cdot L^{-1}$ were prepared and detected in spiked tap water samples. Finally, three types of pesticides, namely, phosmet, chlorpyrifos-methyl and fenthion standard solutions (1×10^{-5} $mol \cdot L^{-1}$), were prepared for the interference experiments.

2.4. SERS Test

The Raman instrument was preheated for 30 min to obtain stable signals before testing. The process of detecting malathion is as follows: 20 μL AgNPs@Sp, 20 μL aptamer-malathion solution, and 10 μL 0.1 $mol \cdot L^{-1}$ aggregate (NaCl, KCl or $MgCl_2$) were mixed well after 5 min for the SERS test. The laser spot focused on the surface of the solutions by adjusting the depth and location of the laser beam with a focus lens. The SERS spectra were recorded by scanning from 400 cm^{-1} to 2000 cm^{-1} with a 30 s integration time and double integrations.

3. Results and Discussion

3.1. SERS Detection Mechanism

Fig. 1 shows the detection mechanism scheme of the proposed SERS sensor for specific detection of malathion. First, the AgNPs were modified by spermine to form AgNPs@Sp. The AgNPs@Sp colloids reportedly formed highly stable suspension because of the positive spermine [27]. Furthermore, the aptamer specifically captured the malathion and formed aptamer-malathion. Because the negative phosphate backbone [28] of the aptamer can be combined with the positive spermine by electrostatic interaction. The aptamer-malathion adsorbed on the surface of AgNPs@Sp. And then, the characteristic peak of the binding of aptamer with malathion was recorded. The relationship of the characteristic peak intensity with the concentration of malathion was established. Therefore, the label-free apatamer sensor was developed for pesticide malathion residues.

3.2. Characteristic of Ag Colloids

AgNPs reportedly exhibit apparent surface plasma resonance (SPR), which depends on the composition, size, and shape of the Ag NPs [29]. From Fig. 2(c), a strong absorption peak appears at 418 nm and shows relatively narrow distribution. Generally, narrower half-width means a narrower particle size distribution [30]. From the SEM spectra (Fig. 2(a, b), the shapes of AgNPs are mostly spherical. Fig. 2(d) shows the particle size statistics and percentage distribution of AgNPs. The distribution of the AgNPs is similar to that of normal distribution. It can be seen that the particle size is mainly distributed in the range of 37–60 nm, which indicates that the particle size distribution of AgNPs is uniform and relatively concentrated, so it can meet the requirement of the homogeneity and consistency of the substrate.