Subnanomolar detection of ochratoxin A using aptamer-attached silver nanoparticles and surface-enhanced Raman scattering

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We report a new approach for the detection of ochratoxin A (OTA) by introducing surface-enhanced Raman scattering (SERS). This method is based on different adsorption propensities of Raman reporter Cy5-tagged OTA aptamer. The OTA aptamer could be easily adsorbed onto the surface of silver nanoparticles (AgNPs) to give fairly strong SERS signals, whereas such bindings would be hampered in the presence of OTA. On the addition of OTA in the concentration range of 0.1–10 nM, the SERS signals appeared to decrease by ~40%, since OTA’s aptamer could not adsorb onto the surface of AgNPs due to binding with OTA. We verified our results against a control experiment with warfarin, which did not affect the SERS signals. A nanomolar detection limit of OTA was achieved using the current SERS-based method.

Introduction

Raman spectroscopy has been extensively used in analytical methods. Surface-enhanced Raman spectroscopy (SERS) has been used recently to detect trace amounts of pathogen oligonucleotides. By combining aggregation-dependent noble metal nanoparticles (NPs) and probe oligonucleotides, ultra-sensitive detection of hazardous compounds and toxins can be achieved in the field of nanobioanalysis. Recent developments in the detection and quantitation of fungal toxins, which are produced by fungi in a variety of commodities, have received much attention from researchers. Raman spectroscopy is currently used to detect mycotoxins.

Ochratoxin A (OTA) is classified as a possible human carcinogen. It is found in cereals, beans, dried fruits, coffee, beer, and wine and is also produced as a secondary metabolite by Aspergillus and Penicillium spp. Various analytical techniques have been employed for the quantification of OTA such as thin-layer chromatography-fluorescence detection and high performance liquid chromatography (HPLC)-fluorescence detection. The approaches based on HPLC techniques, however, require sophisticated equipment and trained personnel. Immunoassays based on antigen-antibody reactions, such as enzyme-linked immunosorbent assays developed for detecting OTA in food stuffs, require a high regulatory cost of antibodies.

Aptamers, which are single stranded oligonucleotides selected in vitro, have been used to provide diagnostic tools for analytes and biotoxins. Aptamers that bind to OTA were identified in 2008. These identified aptamers exhibited a high level of binding specificity to OTA, which was revealed by a lack of affinity to structural analogues of OTA such as warfarin. Note that fluorescence-based OTA sensors use a fluorescence dye-tagged aptamer.

Rothberg’s group reported a novel DNA biosensor using unmodified AuNP colorimetric biosensing based on the distinction of electrostatic properties between single- and double-stranded oligonucleotides (ssDNA and dsDNA) on nanoparticles. By using this propensity, a colorimetric detection method of OTA was also reported using unmodified AuNPs as an indicator and its aptamer as a specific recognition element. Despite several reports on OTA detection using chromatographic and immunological assays, there has been no report on an analytical method using nanoparticle-based Raman spectroscopy. Our new approach may be helpful to develop a new method of monitoring OTA with the benefit of low cost and fast detection. In this communication, SERS was implemented to confirm the dissimilar adsorption behaviors to yield a difference in intensity. We report an OTA detection method using aptamer-modified silver nanoparticles (AgNPs) and Raman spectroscopy.
Materials and methods

OTA and warfarin were purchased from Sigma Aldrich (St. Louis, USA). The aptamer (99%) was provided from Bioneer (Daejeon, Korea). Citrate stabilized silver colloidal substrates were synthesized by following the recipes in the literature. The AgNP diameters were ~50 nm according to the TEM and QELS measurements. The pH value of the AgNP solution was 7.1.

UV-Vis absorption spectra were obtained using a Mecasys spectrophotometer. To further check the adsorption behaviour, SERS measurements were performed on AgNP surfaces. In a previous report, warfarin was used as a control for OTA detection. Fig. 1 shows the schematic diagram of OTA detection by means of SERS. Upon the addition of OTA, the conformation of OTA aptamer is changed from a random coil structure to a G-quadruplex structure.

DNA stock solutions were prepared by dissolving the OTA oligonucleotide aptamer in 10 mM PBS buffer solutions containing 1 mM MgCl₂. These stock solutions were stored at 4 °C prior to use. The positively charged spermine was used to immobilize the aptamers onto negative charged AgNP surfaces. In order to obtain SERS spectra, 1 μL of 10 mM spermine was added to 100 μL of AgNP solution containing 5 nM OTA aptamer. The SERS spectra were recorded after equilibration of the mixtures for 3 min. To perform an OTA detection experiment, an OTA (or warfarin) solution of different concentrations was added to the OTA aptamer solution, and the mixture was equilibrated for 10 min. A volume of 1 μL of a 10 mM spermine solution was added to induce aggregation after mixing the solution with a volume of 100 μL of the AgNP solution. The SERS spectra were recorded after 3 min.

Results and discussion

The UV-visible absorption spectra of AgNPs are shown in Fig. 2(a). By introducing spermine, the absorption band redshifted to indicate particle aggregation, making the SERS detection feasible. Fig. 2(b) shows the QELS data of the AgNPs. The initial size of ~50 nm increased to ~800 nm. The increased mean diameters were almost identical for OTA and warfarin. The standard deviations of the QELS measurements for OTA were much larger than those of warfarin. This may suggest that there is a fluctuation in size due to binding of the aptamer and OTA, whereas warfarin would not affect significant deviations in diameter. Fig. 2(c) and (d) shows the TEM image of AgNPs in the presence of the aptamer. On the basis of these physical characteristics, we performed SERS of OTA on the aptamer-modified AgNPs.

Cy5 dye-labelled OTA aptamer oligonucleotide would not directly adsorb onto the surface of citrate-stabilized AgNPs because they both are negatively charged. An additional counter-charged agent is required to immobilize the oligonucleotide. We used spermine for this purpose. It has been reported that the positively charged spermine may interact strongly with DNA backbone to induce aggregation of citrate-stabilized AgNPs via the counter-charged reagent to enhance the SERS intensity.

Either OTA or warfarin alone does not appear to affect the aggregation condition of AgNPs. On the other hand, regardless of the presence or absence of OTA or warfarin, the addition of spermine clearly induces aggregation in the colloidal solution, as evidenced in the UV-visible absorption spectra. QELS measurements also agreed well with the UV-visible absorption spectra to support the observation of spermine-induced aggregation under our experimental conditions.
Fig. 3(a) and (b) show the SERS spectra of the Cy5-labelled aptamer on AgNPs in the presence of OTA and warfarin, respectively. The SERS spectrum of Cy5 appeared analogous to that recently reported. SERS intensities decreased by ~40% as the OTA concentrations increased from 0.1 nM to 10 nM, whereas the intensities changed minimally by warfarin regardless of the concentrations. As shown in Fig. 3(c), the spectral intensities were more sensitive in the range of 0–1 nM. Above 1 nM, the spectral change was relatively small. This SERS detection limit appeared to be more sensitive than other measurements of OTA.

Although we attempted to obtain the Raman spectra of aptamers in the presence of OTA and warfarin in Aspergillus ochraceus, we could not observe the SERS intensities, presumably due to the interference of the cell culture media. Although further study is necessary to optimize SERS signals, our Raman method may provide a new detection scheme for the mycotoxin OTA.

Our Raman detection may provide a fast and cost-effective method to detect OTA in a convenient way. A clear discrimination between OTA and the control (warfarin) can be achieved by comparing the SERS intensities of Cy5-labeled aptamer immobilized on AgNPs within 30 min. Moreover, there is no need for the laborious sample preparation that is required in other chromatographic methods. High cost of antibodies in the immunological method may be avoided by introducing the relatively inexpensive nanoparticle substrates.

We have to mention that the SERS spectral intensities were relatively unchanged in the high concentration region. Considering that the sensitive linearity range is 0.1–1 nM, our method should be preferably applied to focus on the sub-nanomolar concentration region of OTA.

Compared to the previous report on the microchannel SERS 2D platform for the aptamer detection of OTA, our method has several advantages such as simple sampling procedure and low cost of the substrate preparation. The aqueous colloidal AgNPs may be easily applied to detect mycotoxins both in vitro and in vivo.
Conclusions

We report a new approach for the subnanomolar detection of OTA using SERS that could exhibit detection limits as low as 0.1 nM. This approach is based on conformational change of the Raman reporter-tagged OTA aptamer. In the absence of OTA, the OTA aptamer can be easily adsorbed onto the surface of metal NPs and give good SERS signal; however, in the presence of OTA the OTA aptamer cannot be adsorbed onto the surface of NPs, leading to a decrease in the SERS signal.

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