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Detection of the mycotoxin citrinin using silver substrates and Raman spectroscopy

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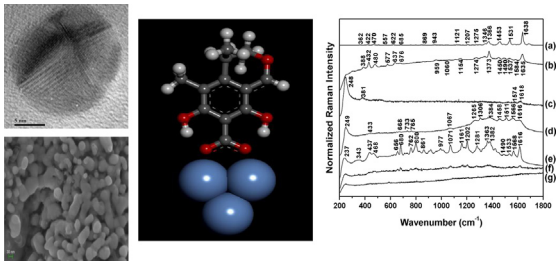
HIGHLIGHTS

- The mycotoxin citrinin was detected using Ag substrates and Raman spectroscopy.
- Prepared Ag substrates were characterized by electron microscopic tools.
- Density functional theory calculation predicted the most stable geometry on Ag.

GRAPHICAL ABSTRACT



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ABSTRACT

We detected a trace amount of the mycotoxin citrinin using surface-enhanced Raman scattering (SERS) on silver nanoparticle (Ag NP) surfaces. The SERS substrate on hydrophobic Teflon films was also introduced to observe the citrinin peaks. A broad band at ~1382 cm^{-1}, which was ascribed to the symmetric carboxylate stretching mode, was observed in addition to an antisymmetric carboxylate stretching mode at ~1568 cm^{-1} in the Raman spectra. The spectral feature indicated that citrinin would adsorb on Ag NPs via its carboxylate form. Based on density functional theory (DFT) calculations, vibrational mode analysis was performed to compare the Raman spectra of citrinin. DFT calculations also predicted that a bidentate bridge configuration through O15 and O16 atoms in citrinin would be the most stable on three Ag atoms. After treating with Ag NPs, observation of citrinin peaks was attempted in fungal cells of *Penicillium citrinum*. This work may provide useful insights into the direct observation of the hazardous citrinin mycotoxin using SERS by understanding its adsorption behaviors on Ag surfaces.

1. Introduction

Research has paid much attention toward the detection of mycotoxin because of its effects in environmental and health issues [1,2]. Citrinin is a nephrotoxic contaminant produced by several fungal species, including *Penicillium* and *Aspergillus* [3,4]. Toxicologically, citrinin is associated with harmful synergistic

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effects, such as induction of DNA damage in cultured renal cells [5]. This toxin has been found to cause the environmental and health problems in a variety of agricultural commodities [6].

Structural information is useful in estimating the toxicity of mycotoxins [7]. Theoretical studies have provided helpful insights into the stability of chemical structures and properties of mycotoxins [8–10]. Because of the unique chemistry of citrinin, it has been the subject of several experimental structural investigations [11]. The molecular structure of citrinin has not been studied extensively, however, despite the relatively simple conformation with its low molecular weight. The *p*- and *o*-quinine methide tautomeric equilibrium of citrinin [11] was calculated using the quantum mechanical method [12].

There have been several analytical methods [13] such as LC–MSMS [14], flow cytometric immunoassay [15], amperometric detection [16], and microfluidic electrochemical immunosensors [17] in order to detect citrinin. Because these methods require time-consuming and expensive sample pretreatment and derivatization steps by a trained expert, it is necessary to develop time- and cost-effective method for the detection of citrinin.

Surface enhanced Raman scattering (SERS) can detect a trace amount of organic hazardous contaminants with a high sensitivity [18–20]. SERS has the advantage of clarifying chemical identity of biomolecules and environmental pollutants adsorbed on metal surfaces. The analysis of spectral features has provided detailed information at high resolution on interfacial adsorption structure and surface reactions. Recently, SERS was applied to detect mycotoxins [21] in combination with density functional theory (DFT) calculations [22].

In this study, we report the detection of the citrinin by using SERS. DFT calculation was also performed to estimate the binding energies to determine the adsorption structures on metal nanoparticle surfaces. The experimental results will be helpful in developing a new scheme for the detection of mycotoxin.

2. Materials and methods

2.1. Preparation of Ag NPs

Citrinin (≥98%) and spermine (≥97%) were purchased from Sigma Aldrich. The other used chemicals were reagent-grade. To prepare Ag NPs, 1 mL sodium citrate (0.57 g/50 mL) is added to ~50 mL the boiling solution of 1.1 mM Ag nitrate stirring on a hotplate [23].

We made a 10^{-2} M stock solution of citrinin in ethanol, which we then used to perform SERS measurements in the presence of metal NPs. For this purpose, the 1 μL volume of 10^{-3} M citrinin solution was mixed with 100 μL of Ag NPs (the final concentration was 5×10^{-6} M) and allowed to react for 3 min. We then mixed 1 μL volume of 10^{-2} M aggregating agents of sodium hydroxide (NaOH), sodium chloride (NaCl), or spermine. After 3 min, we performed the SERS measurements. The round septum polytetrafluoroethylene (Teflon) film made with a radius of 4 mm was purchased from Shimadzu to prepare the SERS platform by a drop-casting method [24]. The SERS-active film was also generated by eroding the Ag plate via HNO_3.

2.2. Physical characterization

The morphologies of Ag NPs were checked using a JEOL JEM-3010 transmission electron microscope. The hydrodynamic radius and the surface potential were measured with an Otsuka ELSZ-2 analyzer. Raman spectra were obtained using a Renishaw Raman confocal system model 1000 spectrometer equipped with an integral microscope (Leica DM LM) attached with a dark-field microscopy set-up [25]. The pH value of Ag NPs was measured by a Thermo Orion 3 benchtop pH meter. The 632.8 nm radiation from a 20 mW air-cooled He–Ne laser (Melles Griot Model 25 LHP 928) was used as the excitation source for the Raman experiments. A data acquisition time of approximately 30 s was used in the Raman measurements to detect citrinin. The scanning electron microscopy (SEM) images of the Ag plates were obtained using a Carl Zeiss Sigma microscope.

2.3. Quantum mechanical calculations

DFT calculations were performed using a Gaussian 03 package. Geometry optimization of the citrinin adsorbate on Ag atom complexes was carried out at the level of B3LYP. LANL2DZ basis sets were used to model the metal atoms. To predict the vibrational frequencies of citrinin in the gas phase, the basis set of 6-31 + G(d,p) was used. Geometry optimization for the two tautomeric structures of citrinin was conducted starting from many possible orientations as shown in Fig. 1. Three plausible binding sites are considered between silver atoms and oxygens of the carboxyl groups in citrinin.

2.4. Cell culture of NPs in Penicillium citrinum

We purchased *P. citrinum* (KCCM 60384, ATCC 36382) from the Korean Culture Center of Microorganisms (Seoul, Korea). The fungi were grown on potato dextrose agar (PDA) medium containing 200 g of sliced potato, 20 g of dextrose, and 20 g of agar powder. For detection of citrinin in live cells, fungi pre-incubated for 2–3 days were treated with a few drops (~10 μL) of colloidal Ag NPs. After mixing with the mounting media, the fungi species sampled from the spot where Ag NPs were dropped and placed on a spectroscopic slide glass. The sample was subsequently sandwiched by the cover glass. The DFM-Raman experiments of the prepared fungi were performed on the microscopy stage [25].

3. Results and discussion

3.1. Physical characterization of Ag NPs

Fig. 2(a) and (b) shows the TEM image of Ag NPs with a size distribution of 10–100 nm. The high-resolution TEM image of a single Ag NP revealed a multi-crystalline structure. The quasi-elastic light scattering (QELS) measurements of Ag NPs showed that the average diameter of Ag NPs was 52.2(±0.6) nm as shown in Fig. 2(c). Almost 90% of the Ag NPs ranged from 10 to 50 nm according to the number distribution. The pH value of the citrate-reduced Ag NPs was measured to be 8.0 (±0.5). It has been reported that the carboxylic acid may adsorb on the negatively-charged Ag NP surfaces via its carboxylate form [26]. We checked the adsorption of benzoic acid on the same negatively charged Ag NPs. Since the p*K*a value [27] of benzoic acid is reported to be 4.17, it is also expected to have anionic forms at the neutral pH state of Ag NPs. The strong symmetric carboxylic stretching band was observed at ~1373 cm^{-1} in their SERS spectra along with the Ag–O band at ~248 cm^{-1}. The obtained SERS spectra indicated a binding of the carboxylic acid molecules on the Ag NPs. Referring to these data, despite the same negative charges, it is probable that the carboxylate may adsorb on Ag due to the chemical binding via either its oxygen lone pairs or the electron donation from metal to its antibonding π^* orbital [26]. This may be evidenced by the observation of the strong metal-oxygen band at 200–250 cm^{-1} in the SERS spectra [28]. On the other hand, the aromatic ring of citrinin may interact with the Ag surfaces via its π orbital. According to the accumulated data, citrinin may adsorb on the Ag NPs with the negative charge.