Surface-Enhanced Raman Scattering from Photoreduced Ag Nanoaggregates on an Optically Trapped Single Bacterium

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A single bacterium in an aqueous Ag nitrate solution was optically trapped by focusing a near-infrared laser beam, and then Ag nanoaggregates were formed on the bacterium by focusing a green laser beam at the bacterium. We obtained surface-enhanced Raman scattering signals from a focal area on a single bacterium.

Conventional Raman spectroscopy favors detection of biological and medical species in aqueous environments, because visible light is insensitive to vibrational modes of water molecules.[1] Hence Raman spectroscopy enables us spectral multiplexing because the vibrational bands especially in the fingerprint region are sharper than fluorescence ones. Furthermore, Raman spectroscopy armed with surface-enhanced Raman scattering (SERS) spectroscopy is sensitive enough for measuring a Raman spectrum of single molecules adsorbed on a nanojunction of a noble metal nanoparticle aggregate (nanoaggregate). The ultrasensitivity is realized by coupling of both incident and Raman light with plasma (plasmon) resonance of the nanoaggregate.[2] SERS is almost free from fluorescence and photobleaching thanks to fast energy transfer from electronically excited molecules to metal surfaces.[2] Recently, SERS spectroscopy has been applied to detect protein molecules on living cells, such as bacteria.[3–9] For example, it has been reported that SERS spectral data discriminate between bacteria to the strain level.[4] In the previous studies, bacteria were coated with Ag by two methods: (1) simply mixed with an Ag colloidal solution;[3–5] (2) soaked in an aqueous Ag nitrate solution, which was reduced to Ag nanoparticles with sodium borohydride.[6–9] SERS spectra have been observed from an ensemble of the Ag nanoparticle-coated bacteria in the previous studies.[3–9] Such ensemble measurements simply provide SERS spectra averaged over many bacteria, and thus discrimination of bacteria between themselves without cultivation and screening them is difficult to do.

To raise the issue in ensemble measurements we measure SERS from single cells, thus giving us a possibility of in situ discriminating between pathogenic and nonpathogenic bacteria without cultivation and screening, during which we have concern about infection and contamination. From a single yeast cell, variations in Raman and SERS spectra using Ag nanoparticle dimers have been discussed in terms of inhomogeneous protein distribution on the cell wall.[10–12] Recently, Raman spectroscopy has been combined with optical trapping, which can avoid undesirable perturbation due to immobilization of a cell on a glass surface, in the single cell measurements.[13] In the case of SERS however, plasma resonance of Ag nanoaggregates coupled with the optical trapping light likely disturbs the measurement. Thus, it is important for single cell spectroscopy to catch a single cell in the suspension independent of synthesizing SERS-active Ag nanoaggregate on the cell wall.

Photoreduction at a laser focal point on a glass surface in an aqueous Ag nitrate solution has already been reported as on-demand synthesis of SERS-active Ag nanoparticles.[14,15] In the present study, the photoreduction was combined with optical trapping of a single bacterium; namely, SERS from a photoreduced Ag nanoaggregate on a single optically trapped bacterium was measured by alternatively irradiating coaxially focused green and near-infrared lasers at the same position. Moreover, we observed a temporal change in SERS spectra from a single bacterium under the continuous irradiation of a green laser on the bacterium. The change was discussed in terms of inhomogeneous SERS-active molecular distribution whose direction is perpendicular to the cell wall planes, namely between surface and inside of the cell wall.

The bacteria *Escherichia coli* JM109 were centrifuged at ≈10000*g* for 5 min, rinsed in water, and recentrifuged; and then suspended in a 1 mM aqueous solution of Ag nitrate. An aliquot of the sample suspension was dropped on a slide glass. This slide glass, on which the bacteria suspension was prepared, was sandwiched with a coverslip to protect the solution against evaporation at room temperature (≈20 °C) as depicted in Figure 1a. The details of our experimental setup were described elsewhere.[15,16] Briefly, a single bacterium was optically trapped by focusing a near-infrared (NIR) laser beam ($\lambda = 1064$ nm, 100 mW) through an objective lens (LCPlanFl

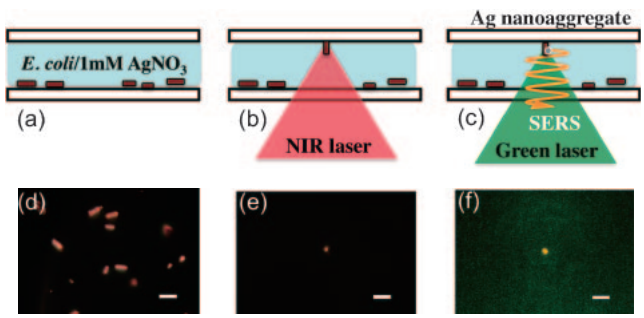

Figure 1. Schematics of experimental procedures for SERS measurement from a Ag nanoaggregate on a single bacterium. (a) Before irradiation of a NIR laser and a green laser. (b) A bacterium was immobilized along its long axis by optical trapping using the NIR laser. (c) Formation of a Ag nanoaggregate on the bacterium by photoreduction of $AgNO_3$ using the green laser. No irradiation of the NIR laser in this procedure. (d)–(f) The dark-field microscope images of *E. coli* corresponding to (a)–(c). Scale bars in (d)–(f) are 10 μm.

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60×, NA 0.7, Olympus) as depicted in Figure 1b. A diode-pumped solid-state green laser beam ($\lambda = 532$ nm, 5 mW) was focused on the optically trapped bacterium through the same objective lens, and then a Ag nanoaggregate was formed by photoreduction as depicted in Figure 1c. The SERS light from the Ag nanoaggregate on the bacterium was collected with the same objective lens and led to a polychromator coupled with a thermoelectrically cooled CCD through a notch filter. Note that photoreduced Ag nanoaggregates covering the entire area of the focal point enable us efficient SERS detection. The required accumulation time for SERS detection is only 5 and 15 s at the longest, in contrast to several minutes for conventional Raman measurements[10,13] and 30–120 s for ensemble SERS measurements,[3,6,9] in which only parts of the illumination area are covered with Ag nanoaggregates. After the photoreduction, the *E. coli* was dried in a vacuum desiccator, followed by deposition of osmium on the cell surface, and then the osmium-coated *E. coli* was observed with a scanning electron microscope (SEM) (JSM-6700FZ, JEOL).

We observed the optical microscopic images of *E. coli* before and after optical trapping and photoreduction using a dark field illumination. Figure 1d shows that *E. coli* followed random directions on the surface of a slide glass. Figure 1e shows that an optically trapped single *E. coli* was aligned vertically, namely along the laser beam direction. Due to the vertical alignment, position-sensitive synthesis of Ag nanoparticles on *E. coli* was difficult. After trapping a single *E. coli*, we focused the green laser beam on the optically trapped *E. coli*. Figure 1f shows an orange spot at the focal point after a few seconds of green laser irradiation. Note that we turned off the NIR laser just before focusing the green laser beam. After the NIR laser was turned off, the *E. coli* with Ag nanoaggregates was still trapped by the focused green laser and again drifted away after the green laser was also turned off. Simultaneous focusing of both NIR and green laser beams into the sample suspension generated a bubble at the focal point. The bubble disturbed the optical trapping of *E. coli*. The bubble is likely induced by temperature elevation of the photoreduced Ag nanoaggregate whose plasma resonated with the highly intense NIR laser light.[2] Indeed the plasma resonance spectrum of an ensemble Ag nanoparticles (like Figures 2b and 2c) has a broad line width.[17] Thus, the plasma resonance of the photoreduced Ag nanoaggregate can be resonated with the NIR laser light. Unfortunately, the plasma resonance spectrum of the Ag nanoaggregate on an *E. coli* cannot be measured due to disturbance of strong Rayleigh scattering light from the *E. coli*. We checked that *E. coli* without Ag nanoaggregates can be stably trapped by simultaneously focusing the laser beams in water.

We observed an *E. coli* before and after the appearance of the orange spot (Figure 1d) using SEM. Figure 2a shows that an *E. coli* was not adsorbed by Ag nanoaggregates before photoreduction. Figure 2b shows that an Ag nanoaggregate that consists of 10 or more Ag nanoparticles was formed on the cell surface. Figure 2c shows that the Ag nanoaggregate, which looks like a bright spot, broke the outer membrane of the cell wall, and sunk in the cell surface. The Ag nanoaggregate was identified by energy dispersive X-ray (EDX) spectra. Figure 2d shows appearance of a peak at 3.0 keV (Ag) on a bright spots

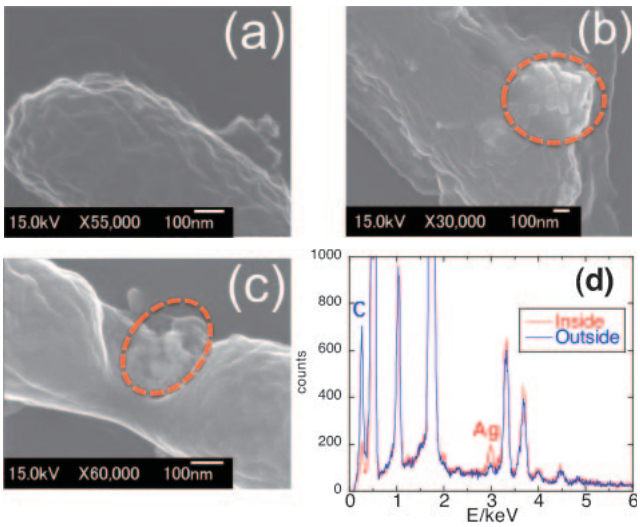

Figure 2. SEM images of *E. coli* (a) before and (b, c) after photoreduction of $AgNO_3$ by focusing a green laser beam on an *E. coli* under the same conditions. (d) EDX spectra inside and outside an orange circle on *E. coli* like (b, c).

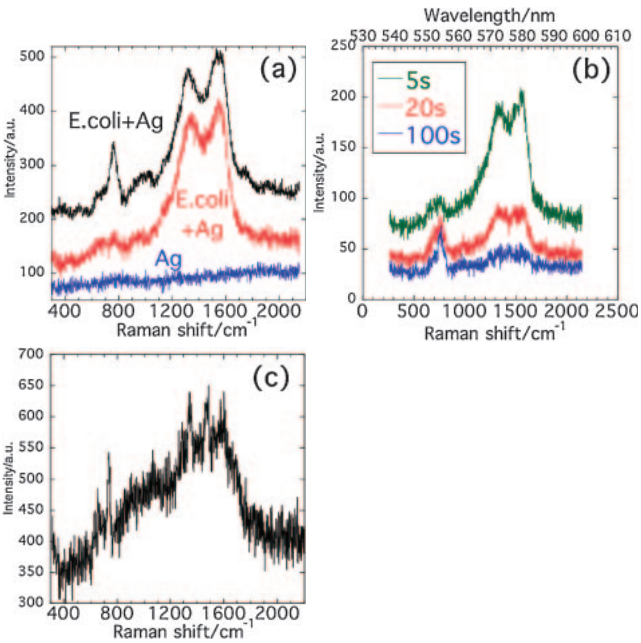

Figure 3. (a) SERS spectra from Ag nanoaggregates on single optically trapped *E. coli* cells (black and red) and on a coverslip (blue) accumulated for 15 s. (b) The time-resolved SERS spectra after green laser irradiation, each of which was accumulated for 5 s. (c) SERS spectrum of cytoplasmic fraction of *E. coli* accumulated for 240 s.

on *E. coli* after photoreduction with a decrease in a peak at 0.25 keV (carbon).

We measured the SERS spectrum from an orange spot (Figure 1f) which may be from a Ag nanoaggregate on a single *E. coli* as indicated in Figures 2b and 2c. Figure 3a shows that two broad peaks were always located at ≈1550 and ≈1300 cm^{-1}, and a sharp peak was often observed at ≈750 cm^{-1}. Note that we did not obtain SERS spectrum from a photoreduced Ag nanoaggregate on a coverslip. Thus, the

SERS signals were obtained from *E. coli* cell. The broad peaks are similar to averaged SERS spectra of amorphous carbon formed by photo- and thermodegradation of carbon monoxide.[18] In each SERS spectrum of amorphous carbon before averaging, however, sharp peaks are observed at random positions[18] unlike the present SERS spectrum in Figure 3a. We confirmed that the present spectrum is similar to a SERS spectrum from *E. coli* in an Ag colloidal suspension at a low concentration of *E. coli* (10^3 cfu mL^{-1}, where cfu is an abbreviation for colony forming units).[3] In Ref. 3 the peaks in the SERS spectrum from *E. coli* (10^5 cfu mL^{-1}) are attributed to Raman bands of flavin adenine dinucleotide (FAD),[3] which exists on the cell surface and plays an important role in respiratory processes of living cells.[8,9] Spectral variations in SERS were not so large compared to previous our results.[11,12] The small variations indicate that we detected rather averaged SERS spectra. Indeed, the observed Ag nanoaggregates are much larger than dimers as shown in Figures 2b and 2c.

Time-resolved SERS spectra were measured from the photoreduced Ag nanoaggregate on the single *E. coli*. During the measurement, we continuously illuminated the *E. coli* with the green laser. Figure 3b shows that the sharp peak at $\approx$750 cm^{-1} became prominent after reduction of the broad peaks at $\approx$1300 and $\approx$1550 cm^{-1}. We consider two possibilities of the changes in the SERS spectra: one is changes in plasma resonance by continuous photoreduction, and the other is detection of two kinds of molecules. The spectral shape of SERS is affected by a change in plasma resonance through the second electromagnetic enhancement due to coupling of Raman light and plasma resonance.[17] With the growth of a photoreduced Ag nanoaggregate, its plasma resonance maxima are preferably red-shifted,[15,16] and thus the Raman peaks in the higher wavenumber region are expected to be enhanced due to the red shifts in plasma resonance maxima. However, this expectation is against the observation in Figure 3b. Thus, the appearance of signal at $\approx$750 cm^{-1} after the disappearance of the broad peaks at $\approx$1300 and $\approx$1550 cm^{-1} may indicate detection of two kinds of molecules. One candidate which exhibits SERS peaks at $\approx$1300 and $\approx$1550 cm^{-1} is the molecules existing on the cell surface (FAD?). Then the other candidate which exhibits SERS peaks at $\approx$750 cm^{-1} is the molecules inside the cell because of penetration of a photoreduced Ag nanoaggregate inside the cell by an increase in its size as indicated in Figure 2c. In the SERS spectrum from an ensemble of Ag-coated *E. coli*, the peak at $\approx$750 cm^{-1} has been attributed to tyrosine or an adenine moiety.[6,9] To confirm the attribution, we observed conventional SERS spectrum of cytoplasmic fraction of *E. coli* from a mixture of a disrupted *E. coli* suspension and a concentrated Ag colloidal solution with 0.5 M NaCl, which facilitates the molecule to adsorb on the Ag surfaces. Figure 3c shows sharp peaks at $\approx$740, $\approx$1340, and $\approx$1470 cm^{-1} in the spectrum. These peaks are attributed to adenine, in particular the prominent peak at $\approx$733 cm^{-1} is due to the ring breathing mode.[19] Thus, the peaks at $\approx$750 cm^{-1} in Figure 3b are likely an adenine moiety inside the cell of *E. coli*. Appearance of three peaks in Figure 3c may indicate that facilitation by dense NaCl solution changes the orientation of adsorbed molecules.

In conclusion, we demonstrated SERS measurements of an optically trapped single *E. coli* by photoreduction of an Ag nanoaggregate. In the SERS measurement, the NIR laser was turned off; the green laser beam was focused to induce resonance of plasma of the photoreduced Ag nanoaggregate on the *E. coli*. The difference in SERS spectra between *E. coli* (Figure 3a) and the changes in the time-resolved SERS spectra (Figure 3b) are likely attributed to amorphous carbon or FAD on the cell and an adenine moiety inside the cell.

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Supporting Information

SEM images and the typical SERS spectra of photoreduced Ag nanoaggregates on various single optically trapped *E. coli* cells. These materials are available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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