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Observation of a Small Number of Molecules at a Metal Nanogap Arrayed on a Solid Surface Using Surface-Enhanced Raman Scattering

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Abstract: In situ Raman spectroscopic measurements with 785 nm excitation were carried out in aqueous solutions containing bipyridine derivatives. Intense Raman signals were observed when the Ag dimer structure was optimized. The SERS activity was dependent upon on the structure of the Ag dimer with a distinct gap distance, suggesting that the intense SERS originates from the gap part of the dimer. Characteristic time-dependent spectral changes were observed. Not only a spectrum which was the superposition of two bipyridine spectra but also spectra which can be assigned to one of the bipyridine derivatives were frequently observed. Observation using solutions with different concentrations proved that the spectra originated from very small numbers of molecules at the active SERS site of the dimer.

1. Introduction

Detection, recognition, and control of single molecules are a common theme in recent advanced technologies. Obtaining vibrational information of single molecules by spectroscopic methods may lead to the discovery of novel phenomena localized on the nanoscale. It has been demonstrated that the surface-enhanced Raman scattering (SERS) phenomenon can drastically increase the scattering cross section, which is comparable to that of fluorescence at high quantum yield.[1−4] Because it provides a wealth of information regarding vibrational modes, chemical structures, and electronic structure of the target molecule, the importance of single-molecule SERS (SM-SERS) has been well recognized from the early stage of its discovery. Despite this, there are only a few examples supporting the observation of SM-SERS. Generally, most of the studies on SM-SERS have been carried out in solution or with deposition of ultralow concentrations to assume that the SERS signal originated from a single molecule.[1−4] The signal blinking observed under these conditions has been regarded as the signature of SM-SERS. Although the polarization dependence[1] and statistically quantized intensity[2] of SERS spectra of this variety may reflect the single-molecular characteristics, blinking is not alone sufficient to demonstrate SM-SERS. Recent efforts on SERS experiments focus on finding additional evidence for SM-SERS.

One of the ways to demonstrate SM-SERS is by analysis of a solution containing more than two kinds of molecules. If one can discriminate one of the molecules from another by spectral analysis, the result may strongly suggest that the SERS active site is active enough to obtain the vibrational information of a small number of molecules in solution. Recently, Le Ru et al. proposed a method to prove SM-SERS via the statistical analysis of SERS spectra from a mixture solution containing rhodamine6G and benzotriazole molecules.[5] The Ag colloidal solution containing the two kinds of molecules showed a spectra dominated by one of the molecules, rather than the ensemble. Dry colloid immobilized onto the surface of polylysine-coated glass also showed similar results. They proposed that the observation is unambiguous proof of SM-SERS.[5] It is noteworthy that the condition of low concentration (1200 molecules per one colloid particle) is not necessary to observe these characteristic spectra. The result suggests that an active SERS site can detect a single molecule, without isolation of molecules. Another observation of blinking behavior in systems of relatively high concentration[6,7] also eliminates the requirements of low concentration for SM-SERS shown in previous studies.

Although the method of observing SERS from a mixture of two kinds of molecules provides an alternative proof of SM-SERS, further careful control of the experimental conditions should be carried out. SERS spectra originate from molecules at the SERS active site, which is possibly highly localized at the surface of optically active metals.[8−11] If one of molecules

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forms an aggregate whose size is larger than the size of the SERS active site, this situation results in the observation of an ensemble spectrum of the aggregate, rather than that of the single molecule. It is a general tendency that molecules with similar chemical structure tend to form aggregates. To avoid the aggregation, surface coverage of the adsorbed molecules should be controlled by the preparation conditions. Furthermore, molecules are known to diffuse on surfaces at room temperature. When two kinds of molecules adsorbe on the surface, the diffusion of molecules of one type into and out of the SERS active site may lead to spectral switching between two kinds of spectra of the molecules as well as the spectral blinking. Considering these processes of molecules at the surface, the experiment to prove the observation of SM-SERS should be carried out via continuous measurement of just one specific active site showing an intense SERS signal under the controlled coverage of two kinds of molecules.

In the present study, well-ordered, periodic Ag nanodot dimer arrays were prepared by adopting an angle-resolved, nanosphere lithography technique (AR-NSL).[12] The gap distance between two Ag dots was optimized to show intense SERS in an aqueous solution containing two kinds of bipyridine derivatives. Confocal spectral and image measurements were carried out by changing the concentration of the bipyridine mixture.

2. Experimental Section

The detailed experimental procedure in the present study was described in our previous report.[13,14] Briefly, a commercial Raman microprobe spectrometer (Ramascope, Renishaw) was specially modified for NIR laser light ($\lambda_{ex} = 785$ nm).[15,16] The expanded NIR beam is focused onto the sample using a water-immersion objective lens with 100× magnification and a numerical aperture of 1.0. The estimated spot size of irradiation, ~1 μm, with tunable output intensity was in the range between 30 μW and 1 mW. Raman image measurement was carried out by imaging Raman scattering at the excitation using an expanded NIR laser beam with a diameter of approximately 30 μm. Extinction spectrum of the Ag dimer array in the visible-near-infrared region were recorded utilizing a multichannel spectrometer (MCPD-2000, Ohtsuka Electronics, observed area was ~0.3 cm^2). The structure of the dimer on the glass substrate was inspected by an atomic force microscope (AFM, Nanoscope-IIIa, Digital Instruments) in air.

3. Results and Discussion

The Ag dot dimer arrays were prepared by AR-NSL[12] using repeated vapor depositions with different angles onto the PS particle ($d = 350$ nm) monolayer prepared on a glass substrate. After the first deposition at 0° in the angle between the surface normal and the Ag deposition beam, the second deposition was carried out at different angles, θ, varying from 15° to 23°. Figure 1a presents typical AFM images of Ag dimer arrays obtained after removal of PS particles. The length of the perpendicular bisectors of the equilateral Ag triangles formed by the first deposition was approximately 130 nm, and the length formed by the second deposition was approximately 80 nm. Directions of the long axis of the individual dimers were well aligned with the azimuth of the second deposition.

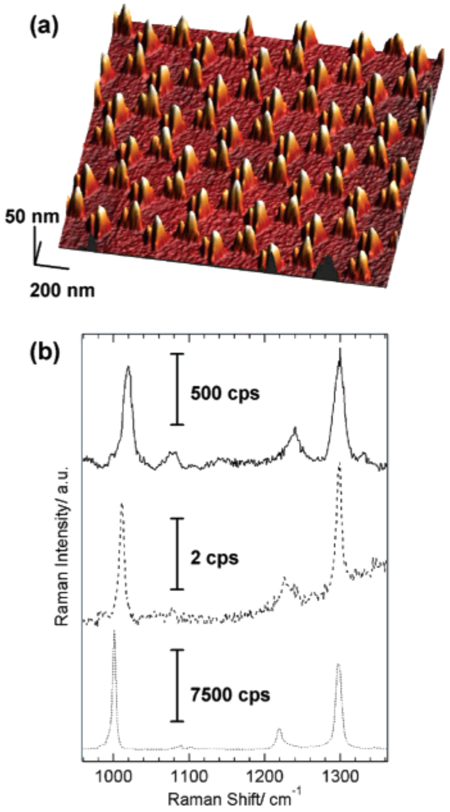

Figure 1. (a) AFM three-dimensional (3D) image of periodic Ag dimer arrays ($d = 350$ nm, $\theta = 18°$). (b) NIR Raman spectra observed in the Ag dimer arrays in 1 mM 4,4′-bipyridine aqueous solution ($W_{ex} = 30$ μW, $t_{ex} = 1$ s; solid line), 20 mM aqueous solution ($W_{ex} = 1$ mW, $t_{ex} = 500$ s; dashed line), and bulk crystal ($W_{ex} = 1$ mW, $t_{ex} = 10$ s; dotted line).

The substrate with the Ag dimer arrays exhibited relatively intense SERS. Figure 1b shows the SERS spectrum measured using an aqueous solution containing 1 mM 4,4′-bipyridine using the Ag dimer arrays. Normal Raman spectra of the bulk crystal and homogeneous aqueous solution under conditions without the enhancement were also shown for comparison. All of the spectra exhibited Raman bands, which can be attributed to the vibrational modes of the 4,4′-bipyridine molecule at 1018 cm^{-1} (ring breathing), at 1080 cm^{-1} (in-plane ring deformation, C−H bending), at 1240 cm^{-1} (in-plane C−H bending), and at 1300 cm^{-1} (inter-ring stretching).[17,18] Enhanced scattering at the dimer array was clearly demonstrated by relatively low excitation power (W_{ex}) and short exposure time (t_{ex}) compared with those observed for bulk crystals and in homogeneous aqueous solutions of relatively high concentration. An empirical signal enhancement factor, which was determined from the peak integration ratio of the SERS vibration at 1018 cm^{-1} to the unenhanced signal from the solution containing 20 mM of 4,4′-bipyridine molecules, was found to be in the range between 10^5 and 10^9.[13,19] It is noteworthy that the present estimation of the enhancement factor is spatially averaged, assuming that the 4,4′-bipyridine molecules homogeneously cover not only the Ag dimers but also the glass surface where negligibly weak

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(19) The number of molecules on the surface was assumed to be in the range between a projection of the amount in solution (10^2 molecules μm^{-2}) and a full coverage of the monomolecular layer (10^6 molecules μm^{-2}).

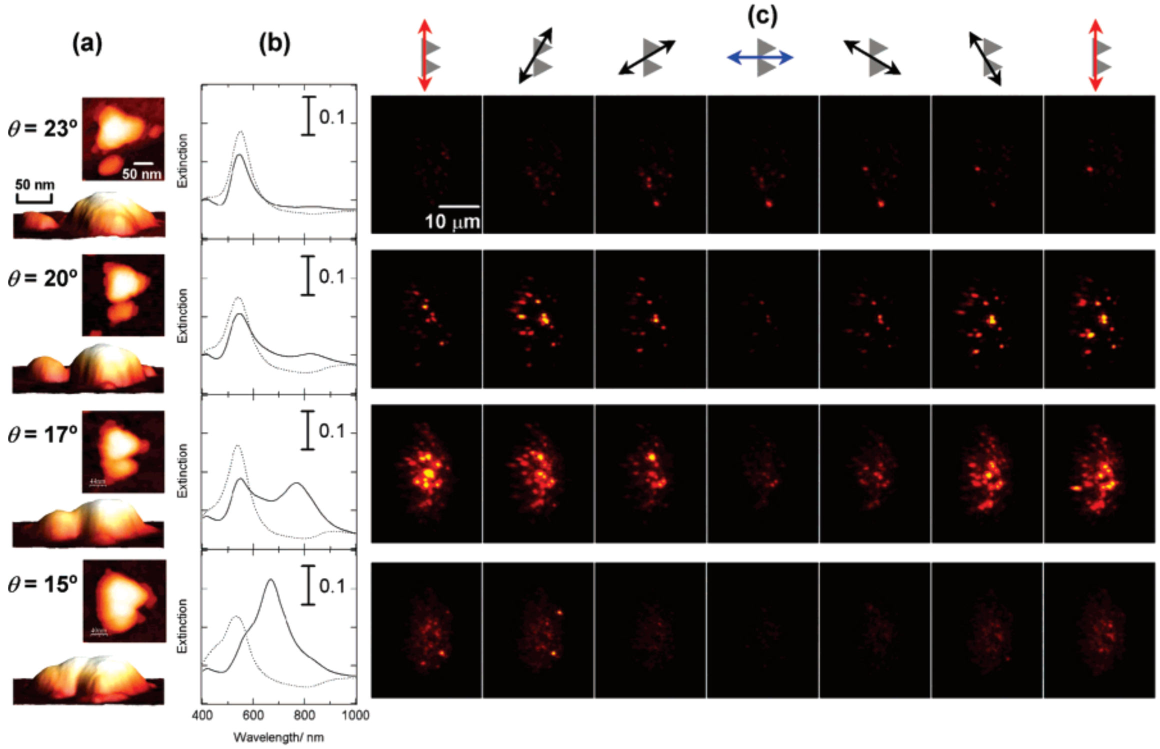

Figure 2. (a) AFM 2D and 3D images of individual dimers at different angles (θ), (b) polarized extinction spectra of Ag dimer arrays (polarization angle to the long axis of dimmers was 0° (dotted line) and 90° (solid line)), (c) NIR Raman images of 4,4′-bipyridine adsorbed on Ag dimer arrays at 1018 cm^{-1} (W_{ex} = 1 mW, t_{ex} = 5 s).

Raman signals may be seen under the present conditions. The enhancement possibly occurs at specific "hot sites" on the Ag dimers;[8−11] thus, it may be several orders of magnitude higher.

To clarify the origin of the intense SERS signals on the Ag dimer structure, the inter-particle distance dependence of the Raman intensity was investigated using various dimer arrays prepared under different θ. Figure 2a shows the typical AFM images of four kinds of Ag dimers fabricated with $\theta = 23°$, 20°, 17°, and 15°. For the dimer prepared at 23°, the inter-particle distance was more than 10 nm. The distance decreased with the decrease of θ. Two Ag particles connected to each other, forming a dimer structure at $\theta = 17°$ and 15°. The AFM image showed that the structure of the dimer became an anisotropic particle rather than a two-triangle dimer at the substrate prepared at $\theta = 15°$. Figure 2b shows the extinction spectra of the periodic Ag dimer arrays measured by the polarized incident light. When the polarization of the illuminated light coincided with the short axis of the Ag dimers, the only peak originated from the localized plasmon excitation at around 520 nm. On the other hand, the polarized excitation along the long axis led to the appearance of a peak at longer wavelengths (600−800 nm), in addition to that at shorter wavelengths. The extinction in the longer wavelength region became stronger as θ decreased. Evolution of the band at the longer wavelength qualitatively agrees well with the behavior at previous theoretically calculated spectra of "tip-to-side" Ag triangular dimers whose distance decreases by a few nanometers.[9] Observed spectral changes with θ suggest that the inter-particle distance of the Ag triangular dimers were controlled in the same size range at the present system. It is noteworthy that these extinction spectra were found to be independent of the observed area within ~1 cm^2. The result proves the successful preparation of uniform and well-ordered structures of the Ag dimer arrays with relatively large areas.

Raman images of the scattering at 1018 cm^{-1} (ring breathing mode) are represented in Figure 2c. Many bright spots in the Raman scattering were observed, especially at the substrate prepared at $\theta = 17°$. The intensity of the spots was dependent on the polarization angle of the excitation. When the polarization direction aligned to the long axis of the Ag dimer array, the intensity of the bright spots of Raman scattering became maximized. Intensity became weak when the polarization direction was close to the perpendicular direction to the long axis of the dimer. Images of the spots, as well as the dependence of the polarization angle, were less apparent at the substrates prepared at $\theta = 23°$, 20°, and 15° than those at $\theta = 17°$. These characteristics of the Raman images showed a correlation to the extinction intensities at $\lambda_{ex} = 785$ nm shown in Figure 2b. The substrate prepared at $\theta = 17°$, having the extinction peak close to λ_{ex}, showed the most intense Raman scattering. It has been suggested that the strongest enhanced field is localized at the junction between the aggregates of nanoparticles.[8−11] Therefore, in our results, the sites showing strong Raman signals should correspond to the gap of the two triangular Ag particles having an appropriate distance. Although the extinction intensity at $\lambda_{ex} = 785$ nm of the substrate prepared at $\theta = 15°$ was comparable to that at $\theta = 17°$, completely fused triangles did not show intense Raman scattering. This result also supports the importance of the gap distance for the formation of the Raman hot spot.[20]

To obtain information on the active SERS site, confocal spectral measurements were carried out using the substrate with

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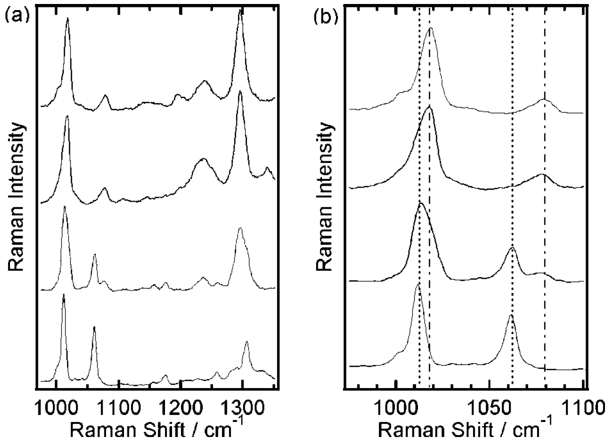

Figure 3. SERS spectra observed at the periodic Ag dimer array in the aqueous solution mixtures of 4,4′-bipyridine and 2,2′-bipyridine (100 μM solution mixture (top black line); 1 μM solution mixture (second black line), comparing typical SERS spectra of 4,4′-bipyridine (top gray line) or 2,2′-bipyridine (bottom gray line). All spectra were recorded with a time of 60 s (except for the 100 μM mixture solution of 200 s) at 30 μW excitation (except for pure 2,2′-bipyridine at 140 μW); a wide range (a) and a narrow (b) wavenumber range.

the Ag dimer prepared at $\theta = 17°$. Figure 3 represents the SERS spectra of the aqueous solution mixture of 4,4′-bipyridine and 2,2′-bipyridine measured at $t_{ex} = 60$ s. Relatively long t_{ex} was adopted to obtain averaged SERS spectra, thus minimizing the effect of the spectral fluctuation. Typical SERS spectra of 4,4′-bipyridine and 2,2′-bipyridine obtained under comparable conditions were also shown for comparison. From these spectra of single components of the bipyridine solutions, it was shown that the ring breathing mode (ν_α) and the mode of the in-plane ring deformation, coupled with the C−H bending (ν_β), appear at 1018 and 1080 cm^{-1} for 4,4′-bipyridine, and at 1011 and 1061 cm^{-1} for 2,2′-bipyridine, respectively.[17,18,21] We can discriminate the molecules by use of these wavenumber differences in each mode in 4,4′-bipyridine and 2,2′-bipyridine molecules.

SERS spectra of a solution mixture containing equal amounts of 4,4′-bipyridine and 2,2′-bipyridine molecules showed a significant concentration dependence (two spectra in the middle in Figure 3b). At relatively high concentrations of 100 μM 4,4′-bipyridine and 100 μM 2,2′-bipyridine solution mixture, the SERS spectrum exhibited dominant bands of $\nu_\alpha = 1018$ cm^{-1} and $\nu_\beta = 1080$ cm^{-1}, originating from 4,4′-bipyridine molecules. Despite the fact that the same amount of 2,2′-bipyridine and 4,4′-bipyridine molecules were contained in solution, characteristics of 2,2′-bipyridine bands at $\nu_\alpha = 1011$ cm^{-1} and $\nu_\beta = 1061$ cm^{-1} were not apparent in the 100 μM mixture. In the case of relatively low concentrations of 1 μM 4,4′-bipyridine and 1 μM 2,2′-bipyridine mixture, however, the SERS spectrum became normal as expected, i.e., equivalent contribution of the bands of 4,4′-bipyridine and 2,2′-bipyridine molecules. The spectrum seems to be an ensemble of the bands of $\nu_\alpha = 1018$ cm^{-1} and $\nu_\beta = 1080$ cm^{-1} for 4,4′-bipyridine, and those at $\nu_\alpha = 1011$ cm^{-1} and $\nu_\beta = 1061$ cm^{-1} for 2,2′-bipyridine. Differences in the spectra observed at different concentrations could be explained by the formation of a domain structure of 4,4′-bypyridine molecular layers. Bipyridine molecules form a monomolecular layer on solid surfaces.[22,23] Due to intermolecular interactions of 4,4′-bipyridine molecules stronger than those of 2,2′-bipyridine molecules, 4,4′-bipyridine molecules may tend to aggregate, forming a domain structure on surfaces. Formation of the domain structure also leads to more dominant adsorption of 4,4′-bipyridine than of 2,2′-bipyridine, even in the case of equal amounts of molecules in solution. If the size of the SERS active site is smaller than that of the 4,4′-bipyridine domain, the SERS spectrum could show dominant bands of 4,4′-bipyridine, rather than those of 2,2′-bipyridine. In the case of relatively low concentration, homogeneously distributed 2,2′-bipyridine and 4,4′-bipyridine adsorbed on the surface lead to a SERS spectrum composed of equivalently contributed bands of two bipyridines. The observed concentration dependence can be explained by the differences both in the domain structure and the amount of the adsorption.

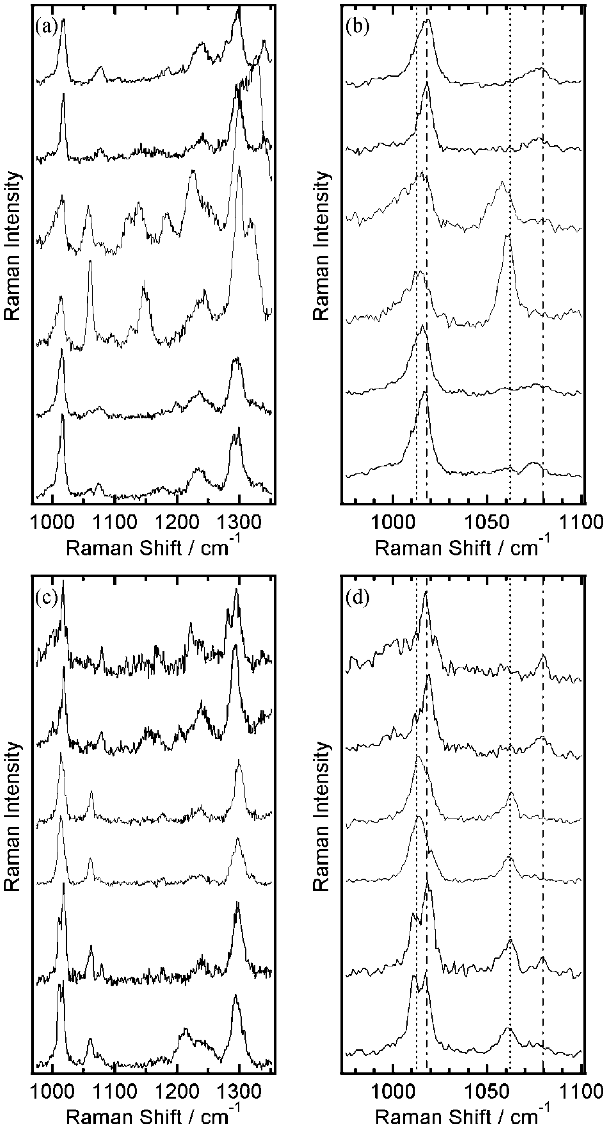

Figure 4. SERS spectra at the periodic Ag dimer array measured in the aqueous solution mixtures of 4,4′-bipyridine and 2,2′-bipyridine in a 100 μM (a,b), 1 μM (c,d) solution mixture. All spectra were recorded with a time of 10 s (except for the third from the bottom of (c, d) 1 s) at 30 μW excitation.

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The present Ag dimer system allows us to obtain the spectra with enough signal-to-noise ratio for the measurement for less than a few seconds t_{ex} because of its relatively high SERS activity. It was found that confocal measurement for short t_{ex} gave completely different spectra from those obtained by the measurements for long t_{ex}. In the cases of both of the 100 μM and 1 μM mixture, the specific spectrum of one of the two bipyridines was clearly observed. Figure 4 shows typical spectra observed for short t_{ex}. During the confocal observation at the same position, spectra showing $\nu_\alpha = 1018$ cm^{-1} and $\nu_\beta = 1080$ cm^{-1} of 4,4′-bipyridine suddenly changed to that showing $\nu_\alpha = 1011$ cm^{-1} and $\nu_\beta = 1061$ cm^{-1} of 2,2′-bipyridine. Although the intensity and the frequency of the appearance fluctuated, spectra showing bands of one bypiridine were well recognized. However, several spectra appeared as a summation of the bands of two bypiridines, similar to the case at long t_{ex} (spectra at bottom in Figure 4); a band at around 1010 cm^{-1} split into two peaks at 1018 and 1011 cm^{-1} for a 1 μM mixture. These peaks correspond respectively to ν_α of 2,2′ -bipyridine and 4,4′-bipyridine. SERS spectra obtained at shorter t_{ex} showed completely different characteristics from those at longer t_{ex}. These results demonstrate that one of the molecules in the mixture can be detected using the Ag dimer, showing relatively intense SERS.

Frequency of the band evolution attributed to the observation that 2,2′-bipyridine was quite low compared to that of of 4,4′-bipyridine in a 100 μM mixture, as apparent in the averaged spectrum of a 100 μM mixture (Figure 3). Although spectral features of 2,2′-bipyridine in a 100 μM solution mixture (middle spectra in Figure 4a and b) were different from others in a single-component solution and in a 1 μM mixture solution, especially in the region between 1100 and 1350 cm^{-1}, these bands can be assigned to the modes of 2,2′-bipyridine shown in previously documented results. The bands at 1146 cm^{-1} (C−H inplane deformation and ring stretching) and 1230 cm^{-1} (ring stretching and C−H inplane deformation) observed at crystalline form shift respectively to 1172 and 1250 cm^{-1} via adsorption.[21,25] Relative intensity of the latter band is known to be sensitive to the molecular conformation. The *cis* isomer showed stronger intensity than the *trans* isomer.[25] Adsorption also leads to the change in the feature of the band at 1300 cm^{-1} (inter-ring stretching). Intensity increase showed the red-shift to be around 1310−1320 cm^{-1}. Based on these assignments, the observed characteristic features of relatively strong bands in the region between 1100 cm^{-1} and 1350 cm^{-1} suggest that the adsorbed 2,2′-bipyridine molecule coordinates strongly to the Ag surface as the *cis* form. Although the origin of relative strong intensities of the bands are not clear at the present stage, high coverage and the interaction with coadsorbed 4,4′-bipyridine in a 100 μM solution mixture may contribute to these vibrational characteristics emerging.

Recently, electromagnetic calculations proved that the SERS enhancement factor can be over 10^{13}, a value which is even high enough to detect single molecules; this is achieved only by the electromagnetic effect at the junction of the two triangular nanoparticles. The present system is also expected to show a comparable enhancement.[9,10] Observed characteristics of SERS spectra at shorter t_{ex} should reflect vibrational information on a very small number of molecules at the SERS active site at the gap of the Ag dimer. Especially in the case of a 1 μM mixture, relatively sharp and distinct characteristics of each spectrum suggest that the system displayed spectra of the single molecules. The bands attributed to 2,2′-bipyridine observed for a 100 μM solution mixture were also quite unique.

Although further detailed analysis of these spectral characteristics may provide information on the adsorbed structure of the molecules, the SERS active site detects a single molecule among two equally distributed bipyridine molecules that are diffusing on the surface of the Ag dimer in solution at room temperature. Generally, the dominant evidence for single molecule detection has been signal blinking, polarization dependency of the spectra, or a Poisson distribution of the signal intensities under extremely dilute concentrations (∼pM). This switching behavior of the molecules may be the novel evidence of single-molecule detection, even under the comparatively high concentration measurement (∼μM).

4. Conclusions

In summary, we have succeeded in optimizing the structure of Ag dimer arrays for the observation of intense SERS. The SERS activity was well correlated to the optical scattering property of the Ag dimer with distinct gap distance, suggesting that the intense SERS originates from the gap part of the dimer. In situ spectral measurements of individual dimers were carried out in an aqueous solution containing equal amounts of 4,4′- and 2,2′-bipyridines. Characteristic time-dependent spectral changes were observed in the present system. Not only a spectrum which is a superposition of two bipyridine spectra but also spectra which can be assigned to one of the two bipyridines were frequently observed. Detailed analysis of the observed spectra using solutions with different concentrations proved that the spectra originated from a very small number of molecules at the active SERS site of the dimer. The frequency of the observation of one of the molecules reflects the amount of the adsorbed bipyridine molecules on the surface. The present observation strongly suggests that optimized structure for intense SERS may have a relatively small size, comparable to that of the molecule, and thus leads to successful observation of individual molecules in complex systems.

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