Identification of Thiacyanine J-aggregates Adsorbed on Single Silver Nanoaggregates by Surface-Enhanced Raman Scattering and Emission Spectroscopy

Yasutaka Kitahama,*[1] Yuhei Tanaka,[1] Tamitake Itoh,[2] Mitsuru Ishikawa,[2] and Yukihiro Ozaki[1]

[1]Department of Chemistry, School of Science and Technology, Kwansei Gakuin University, Sanda 669-1337

[2]NanoBioanalysis Team, Health Technology Research Center, National Institute of Advanced Industrial
Science and Technology (AIST), Takamatsu 761-0395

Received February 9, 2009; E-mail: kitahama@kwansei.ac.jp

By lifting the limitation of ensemble measurement, concentration dependence of surface-enhanced Raman scattering (SERS) and background emission spectra were clearly observed in single Ag nanoaggregates adsorbed by thiacyanine dyes in aqueous solutions (0.5 μM–0.5 nM). Both SERS and background emission spectra at higher dye concentrations characterize the J-aggregates of thiacyanine dyes. On the other hand, no background emission was observed at lower dye concentrations, thereby suggesting that background emission is attributable to J-aggregates of thiacyanine dyes. Furthermore, temporally-stable and -fluctuating SERS spectra, together with the appearance and disappearance of SERS peaks in the 1000–400 cm^{-1} region, are discussed in terms of the J-aggregates and the monomers plus dimers of thiacyanine dyes.

Ionic dyes form supramolecular aggregates called J-aggregates in an electrolyte and concentrated solution due to stacking of their hydrophobic rings.[1] A sharp peak (J-band) in an absorption spectrum red-shifted from the monomer peak characterizes J-aggregates.[1] The optical properties of J-aggregates have widely been studied for practical applications, such as photography, polarizing optics, and nonlinear optics.[1] A reason for the appearance of J-bands is formation of delocalized excitonic states through coherent excitation of many monomers, which results from transition dipole moments of monomers aligned in a straight line.[2–4] Furthermore, a two- or three-dimensional interaction among the aligned excitons induces upper exciton absorption bands and nonlinear optical dynamics in the femtosecond region.[5,6] For these reasons the optical properties of J-aggregates strongly depend on the aggregate structure. J-aggregation, which is similar to crystallization, is influenced by formation and structure of nuclei, namely individual nanosized J-aggregates in the early stage. Thus, it is important to determine the structure of individual nanosized J-aggregates to design and form optical devices involving J-aggregates.

The structure of J-aggregates has widely been investigated by various techniques, such as cryo-TEM (transmission electron microscopy),[7] fluorescence microscopy,[8–13] reflection microscopy,[13,14] AFM (atomic force microscopy),[10–13] X-ray diffraction,[12] and Raman spectroscopy.[15–18] In conventional spectroscopic techniques however, an ensemble of many J-aggregates of various sizes and internal structures is exclusively observed. The use of single molecule or aggregate spectroscopy is effective for exploring nanosized objects one by one. Indeed, fluorescence spectroscopy of individual nanosized J-aggregates in an aqueous solution has already been implemented.[19] In Raman spectroscopy, detailed information about the structure of J-aggregates is provided by peak positions of vibrational modes of the functional groups. However, it is impossible to measure conventional Raman spectra of individual molecules and aggregates due to their extremely small Raman cross sections, ca. 10^{-24} cm^2. To overcome the limitations by the extremely small Raman cross section, surface-enhanced Raman scattering (SERS) spectroscopy has been applied. Indeed, SERS spectroscopy is sensitive enough to measure Raman spectra from individual molecules and aggregates adsorbed on noble metal nanoaggregates,[20–23] because an electromagnetic field is significantly enhanced at a junction in the nanoaggregate.[24] To the best of our knowledge, a limited number of SERS studies of J-aggregates have been published,[25–27] although conventional UV–vis absorption and localized surface plasmon resonance Rayleigh scattering of J-aggregates adsorbed on noble metal nanoparticles have been reported.[28–30] The SERS spectra of 1,1′-diethyl-2,2′-cyanine (pseudoisocyanine, PIC) J-aggregates were observed at limited dye concentrations (24 and 4 μM) and showed peaks at similar positions with and without resonance effects.[25,26] This observation motivated us to change dye concentration to reveal expected differences between the presence and absence of resonance effects. Indeed, the resonance effect involving J-bands looks controllable by extensively changing dye concentration. However, no SERS studies of J-aggregates using various dye concentrations have been reported. Without extensively changing dye concentration and lifting the limitation of ensemble measurement, J-aggregates were not clearly identified in the observed SERS spectra.[25,26]

In the current study, we applied single Ag nanoaggregate SERS spectroscopy to identification of J-aggregates of thia-

Published on the web September 9, 2009; doi:10.1246/bcsj.82.1126

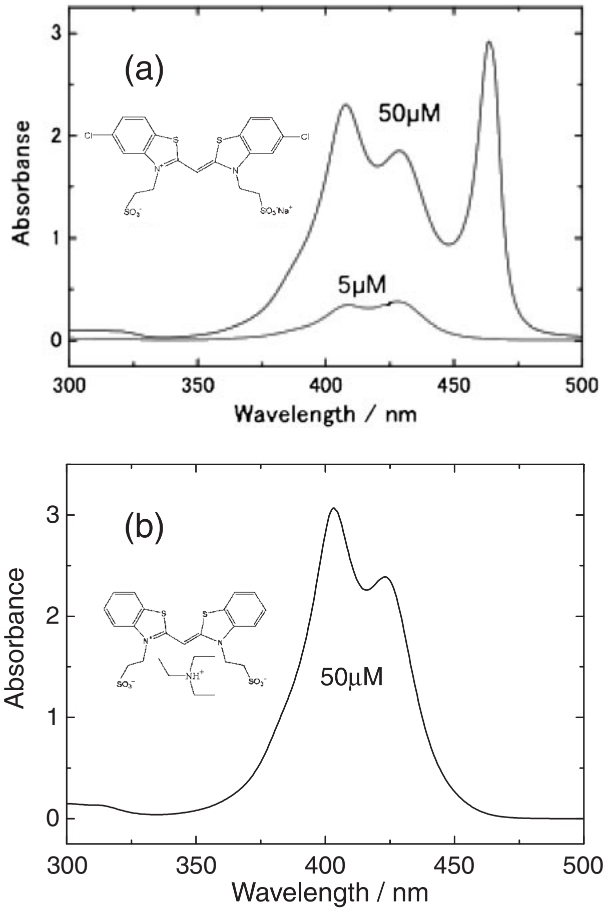

Figure 1. Absorption spectra of (a) 50 and 5 μM TC-Cl_2 and (b) 50 μM TC in a 10 mM NaCl aqueous solution. Insets in (a) and (b) show structures of TC-Cl_2 (5,5′-dichloro-3,3′-disulfopropyl thiacyanine) and TC (3,3′-disulfopropyl thiacyanine), respectively.

cyanine dyes, which are widely known as anionic dyes that form J-aggregates (Figure 1a),[10–13] at various concentrations (0.5 μM–0.5 nM). We successfully distinguished a crucial difference between SERS spectra of thiacyanine adsorbates from a higher concentration solution (0.5 μM) and those from a lower concentration (0.5 nM). The identification of J-aggregates in 0.5 μM adsorbates is principally based on comparison between SERS spectra and known Raman spectra of J-aggregates of thiacyanine dyes in the low wavenumber region (1000–400 cm^{-1}), and additionally comparison between emission spectra from 0.5 μM adsorbates and those from known J-aggregates. In contrast, no observation of Raman peaks in the low wavenumber region and emission spectra from 0.5 nM adsorbates suggests the absence of J-aggregates. The contrasting observations are consistent with previous theoretical and experimental work.[10–13,15–18,26] Additionally, excitation wavelength dependence and temporal fluctuation of the SERS spectra support identification of J-aggregates of thiacyanine dyes.

Experimental

Two kinds of thiacyanine dyes, 5,5′-dichloro-3,3′-disulfopropyl thiacyanine (TC-Cl_2) sodium salt and 3,3′-disulfopropyl thiacyanine (TC) triethylamine, were used as purchased from Hayashibara Biochemical Laboratories (Okayama, Japan). Figure 1 shows the molecular structures of TC-Cl_2 and TC. Colloidal Ag nanoparticles were prepared by the Lee–Meisel method.[31] A stock aqueous solution of thiacyanine dyes (2 μM–2 nM), a NaCl aqueous solution (20 mM), and an Ag colloidal solution were mixed in a volume ratio of 1:1:2. Thus, 0.5 μM–0.5 nM thiacyanine solutions were prepared for sample Ag nanoaggregates, on which thiacyanine dyes were adsorbed. The mixture was incubated overnight at room temperature (ca. 24 °C) and was spin coated on a glass plate, and then the glass plate was rinsed with water and acetone. An aliquot of a 1 M NaCl solution was applied dropwise on the glass plate to stabilize the sample Ag nanoaggregates on the surface. This glass plate, on which sample Ag nanoaggregates were dispersed, was sandwiched with another glass plate to protect the solution from evaporation. Single isolated Ag nanoaggregates were identified by observation of bright spots using a dark-field microscope in white light illumination. The bright spots were characterized by localized surface plasmon resonance Rayleigh scattering lights in various colors.

The experimental details are described elsewhere.[32] Briefly, the sample on an inverted microscope (Olympus, IX-70) was excited using an Ar ion laser (458, 488, or 514 nm). An excitation laser beam was delivered through an angle of 30° against the sample surface using an objective lens (Olympus, UMPlanFl 5×, NA 0.15). The 20 mW laser produced an elliptic spot with a power density of ca. 0.2 kW cm^{-2} on the sample surface. The SERS light from a single Ag nanoaggregate was collected with an objective lens (Olympus, LCPlanFl 60×, NA 0.7) and focused on a polychromator (Acton, Pro-275) coupled with a thermoelectrically cooled CCD (Andor, DV434-FI) through a notch filter and a pinhole. Absorption spectra of thiacyanine dyes were measured with a UV–vis spectrometer (Shimadzu, UV-3101PC). A Raman spectra of J-aggregates in a 1 mM TC-Cl_2 solution containing 10 mM NaCl and that of TC-Cl_2 powder were measured with conventional Raman spectrometers (Jasco, NRS-2100 and Kaiser Optical Systems, HoloLabo 5000) using 514 and 785 nm lines.

Results and Discussion

UV–Vis Absorption. Figures 1a and 1b show absorption spectra of TC-Cl_2 (50 and 5 μM) and TC (50 μM) in a 10 mM NaCl aqueous solution, respectively. Absorption maxima at ca. 410 and ca. 430 nm in Figures 1a and 1b are attributed to the dimers and monomers of each sample.[11] A sharp J-band was observed at 464 nm in the spectrum of a 50 μM TC-Cl_2 solution. However, the corresponding J-band was not observed in the spectrum of a 50 μM TC solution. The formation of J-aggregates in TC-Cl_2 likely depends on electron-attractive Cl atoms rather than steric factors. Indeed, 5,5′-dimethoxy-3,3′-disulfopropyl thiacyanine sodium salt, which includes electron-donating methoxy groups instead of Cl atoms in TC-Cl_2, did not form J-aggregates in an electrolyte solution (data not shown). Thus, TC-Cl_2 forms J-aggregates in the concentrated solution. However, only the monomer and the dimer exist in the concentrated solution of TC and in the diluted solution of TC-Cl_2.

Background Emission and Raman Peaks in the Low Wavenumber Region. Figures 2a and 2b show a low-resolution SERS and background emission spectra of a single Ag nanoaggregate adsorbed from 0.5 μM TC-Cl_2 solution and

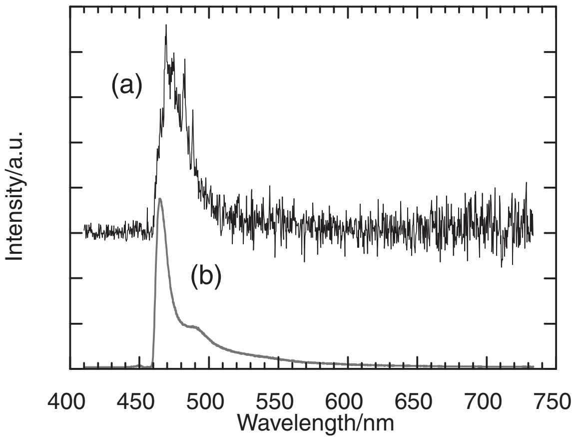

Figure 2. (a) SERS spectrum of a single Ag nanoaggregate adsorbed from a 0.5 µM TC-Cl_2 aqueous solution excited at 458 nm. (b) Fluorescence spectrum of TC-Cl_2 J-aggregates in a 1 mM TC-Cl_2 and 10 mM NaCl aqueous solution excited at 458 nm.

a fluorescence spectrum of J-aggregates in a 1 mM TC-Cl_2 solution, respectively. Excitation at 458 nm generated an emission spectrum (Figure 2a) whose maximum (ca. 470 nm) and band-width (ca. 980 cm^{-1}) are similar to those of the fluorescence of the J-aggregates. Indeed, the fluorescence from such concentrated solution is attributed to J-aggregates of TC-Cl_2.[10–12] The background emission maximum and the band-width in Figure 2a are independent of a plasmon band of a single Ag nanoaggregate (figures not shown) unlike background emissions of SERS spectra in a previous paper[33] and always similar to those of the fluorescence of TC-Cl_2 J-aggregates in Figure 2b.[34] Thus, TC-Cl_2 adsorbates from a 0.5 µM solution are most likely stacked on the surface of a Ag nanoaggregate like J-aggregates in a 1 mM solution and in powder, and thus the SERS and background emission in Figure 3b is attributed to the J-aggregate of TC-Cl_2.

Figures 3a and 3b show a Raman spectrum of J-aggregates in a 1 mM TC-Cl_2 solution; and SERS plus emission spectra of a single Ag nanoaggregate adsorbed from 0.5 µM TC-Cl_2 solution. A Raman spectrum of a 0.5 µM TC-Cl_2 solution was not observed without surface enhancement. The Raman spectrum in Figure 3a is also similar to that of TC-Cl_2 powder (data not shown). This similarity is consistent with the fact that ionic dye molecules like TC-Cl_2 are most likely stacked in J-aggregates like pseudoisocyanine (PIC) bromide.[35] Note that enhanced Raman peaks in Figure 3b were observed at almost the same wavenumbers as those in Figure 3a.

Figure 3c shows a representative SERS spectrum of a single Ag nanoaggregate adsorbed from a 0.5 nM TC-Cl_2 solution. The spectrum is characterized by no background emission in the 1950–400 cm^{-1} region and no Raman peaks in the 1200–400 cm^{-1} region. The SERS spectrum in Figure 3c is clearly different from the Raman spectrum of J-aggregates in Figure 3a. It is noted that in the present study an average spectrum which has characteristics of both spectra in Figures 3b and 3c, for example, the spectrum with background emission and no Raman peaks in the 1200–400 cm^{-1} region, was not observed. Thus, the SERS spectra can be divided into two types like those in Figures 3b and 3c. Decreasing the concentration of TC-Cl_2 from 0.5 µM to 0.5 nM reduced the number of the Ag nanoaggregates that generated the SERS spectrum like Figure 3b. Indeed, the ratio of the Ag nanoaggregates that generated the SERS spectrum like Figure 3b to those that generated the SERS spectrum like "Figure 3c" was changed from 10:0 at 0.5 µM to ca. 3:7 at 5 nM; and finally ca. 1:9 at 0.5 nM.

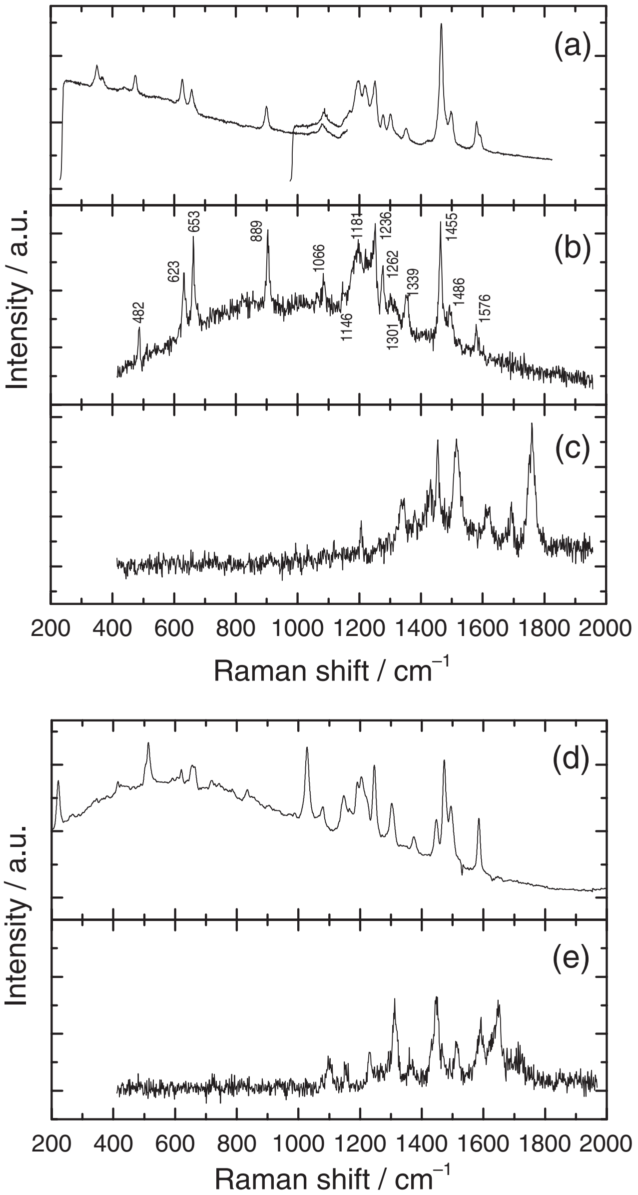

Figure 3. (a) Raman spectrum of TC-Cl_2 J-aggregates in a 1 mM TC-Cl_2 and 10 mM NaCl aqueous solution excited at 514 nm. SERS spectra of a single Ag nanoaggregate adsorbed from (b) a 0.5 µM TC-Cl_2 aqueous solution and (c) a 0.5 nM TC-Cl_2 aqueous solution excited at 458 nm. (d) Raman spectrum of TC powder excited at 785 nm. (e) SERS spectrum of a single Ag nanoaggregate adsorbed from a 0.5 µM TC aqueous solution excited at 458 nm.

Figures 3d and 3e show a Raman spectrum of TC powder and a SERS spectrum of a single Ag nanoaggregate adsorbed from a 0.5 µM TC solution, respectively. Again a Raman

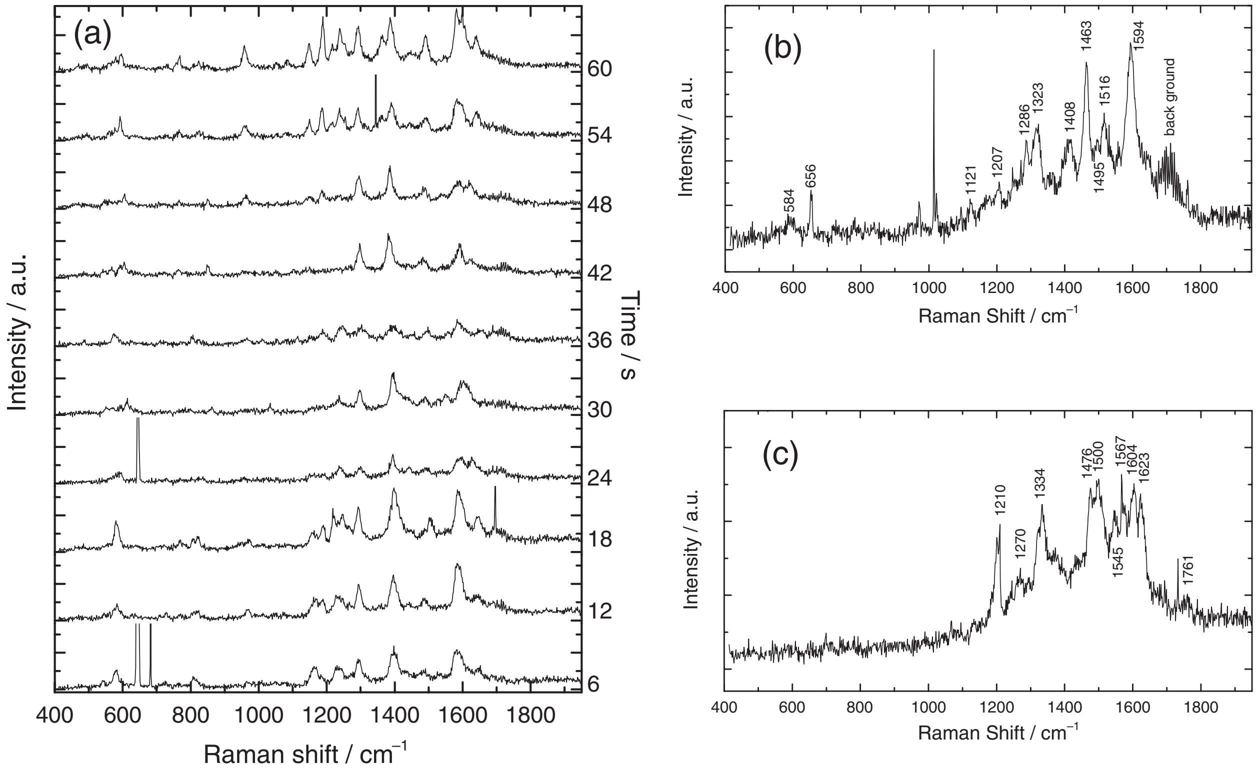

Figure 4. (a) Time-resolved SERS spectra of a single Ag nanoaggregate adsorbed from a 0.5 µM TC aqueous solution excited at 458 nm at intervals of 6 s. (b) Averaged SERS spectrum of a single Ag nanoaggregate adsorbed from a 0.5 µM TC aqueous solution calculated from 60 temporally-fluctuating spectra excited at 458 nm. (c) Averaged SERS spectrum of a single Ag nanoaggregate adsorbed from a 0.5 nM TC-Cl_2 aqueous solution calculated from 22 temporally-fluctuating spectra excited at 458 nm.

spectrum of a 0.5 µM TC solution was not observed without surface enhancement (data not shown). The Raman spectrum in Figure 3d is most likely a Raman spectrum of TC J-aggregates in a similar way that the Raman spectrum of TC-Cl_2 powder is that of the J-aggregates. The SERS spectrum in Figure 3e was totally different from the Raman spectrum in Figure 3d, but similar to the SERS spectrum in Figure 3c in terms of no background emission and no Raman peaks in the 1000–400 cm^{-1} regions, although the concentration (0.5 µM) of a TC solution is much higher than that (0.5 nM) of a TC-Cl_2 solution. Thus, the SERS spectra in Figures 3c and 3e are quite different from the SERS spectra in Figures 3b and 3d. Note that the SERS spectra in Figures 3c and 3e temporally fluctuated. We have already reported time-resolved SERS spectra of TC-Cl_2 molecules adsorbed on a single Ag nanoaggregate.[36] In an aqueous solution, a SERS spectrum with background emission like that in Figure 3b changes into a temporally-fluctuated spectrum without background emission like those in Figures 3c and 3e. It seems that the change coincides with dissolution of a TC-Cl_2 J-aggregate into monomers or dimers.

The SERS spectra in Figures 3c and 3e were not accompanied by the background emission like Figure 3b. No fluorescence of monomer TC-Cl_2 and TC is emitted thanks to the excitation at 458 nm, which is still longer than the absorption of monomers (430 nm) and dimers (408 nm).[11] Note that dimers of TC-Cl_2 and TC are non-fluorescent; and also the fluorescence maxima of monomer TC-Cl_2 and TC appear at 495 nm,[37] which corresponds to 1630 cm^{-1} Raman shift excited at 458 nm. Thus, the background emission in Figure 3b excited at 458 nm, its maximum appears at ca. 1000 cm^{-1}, is exclusively associated with the J-aggregates of TC-Cl_2. A mixture of monomers and dimers of thiacyanine dyes might provide the SERS spectra in Figures 3c and 3e in a similar way that TC-Cl_2 in a 5 µM solution and TC in a 50 µM solution in Figures 1a and 1b, both of which do not show the J-band, are a mixture of monomers and dimers.

In the SERS spectra without background emission, no peaks were observed in the 1000–400 cm^{-1} region (Figures 3c and 3e). The out-of-plane vibrational modes are observed in the 1000–400 cm^{-1} region.[15] Intensities of these modes are enhanced by a factor of 30 through formation of J-aggregates.[15–18,26,27] The enhancement of out-of-plane vibrational modes is theoretically rationalized by activation of out-of-plane vibrational modes of the Albrecht *A* term through resonance Raman effect.[17] Furthermore, such enhancement in the low wavenumber region was experimentally demonstrated in resonance Raman spectra of porphyrin, pseudoisocyanine (PIC), and thiacarbocyanine J-aggregates.[15–18,26,27] Thus, lack of SERS peaks in the 1000–400 cm^{-1} region excludes J-aggregates of TC-Cl_2 and TC in Figures 3c and 3e; rather, allows us to consider the possibility of detecting a mixture of monomers and dimers of TC-Cl_2 and TC in the 1900–1000 cm^{-1} region.

Temporal Fluctuation. Figure 4a shows the 458-nm excited time-resolved SERS spectra of a single Ag nanoaggregate adsorbed from a 0.5 µM TC solution, in which the

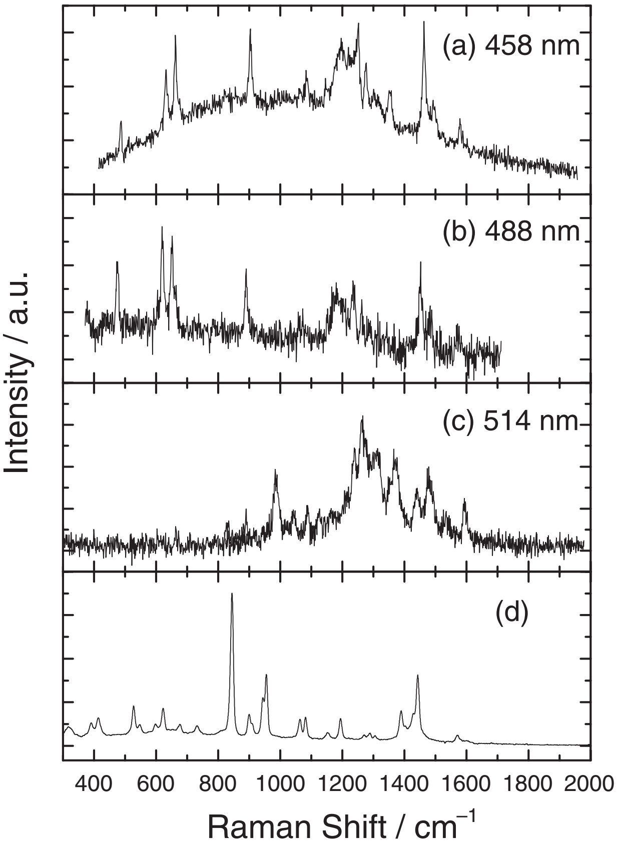

Figure 5. SERS spectra of a single Ag nanoaggregate adsorbed from a 0.5 µM TC-Cl_2 aqueous solution excited at (a) 458, (b) 488, and (c) 514 nm. (d) Raman spectrum of trisodium citrate dihydrate powder excited at 785 nm.

J-aggregates do not exist. Each spectrum was observed at intervals of 6 s. Spectral peaks appeared mainly in the 1000–1700 cm^{-1} region and changed randomly in their intensity and position with time. It has been reported that SERS spectra of R6G temporally fluctuate, however, the blinking SERS peaks did not appear at random positions but appear at similar positions to the corresponding conventional Raman peaks.[21] Temporal fluctuation was also observed from a single Ag nanoaggregate adsorbed from a 0.5 nM TC-Cl_2 solution excited at 458 nm (Figure 3c) and from a 0.5 µM TC-Cl_2 solution excited at 514 nm (Figure 5c), but not observed from a 0.5 µM TC-Cl_2 solution excited at 458 and 488 nm (Figures 5a and 5b). Thus, the disappearance of temporal fluctuation in SERS spectra is most likely due to excitation of J-aggregates from a 0.5 µM TC-Cl_2 solution characterized by the J-band at 464 nm like Figure 1a.

Such temporal fluctuation in the spectral peaks, which is considered as evidence for single molecule detection,[21] was observed even in the use of a high concentration (0.5 µM) TC solution. In this condition, J-aggregates of TC were not formed. Here we consider a possible reason for the temporal fluctuation. We assume that TC-Cl_2 and TC molecules are adsorbed on negatively-charged Ag surfaces by Coulomb attraction through their positively-charged nitrogen atoms like PIC and R6G.[38,39] However, TC-Cl_2 and TC have two negatively charged –SO_3^- groups unlike PIC and R6G. Thus, thermally-driven Coulomb repulsion between the SO_3^- groups and negatively-charged Ag surfaces likely generates temporal fluctuation of SERS spectra of TC-Cl_2 and TC in monomers and dimers at the SERS-active junctions of Ag nanoaggregates. On the other hand, the assumed planar geometry of TC-Cl_2 J-aggregates likely causes stable adsorption of the J-aggregates on the surface of a single Ag nanoaggregate and thus generates SERS without temporal fluctuation, in a similar way that PIC J-aggregates are tightly adsorbed on the Ag surface due to their planar geometry.[40]

To gain an insight into temporally-fluctuated and -stable SERS spectra, we contrast the SERS spectra of three kinds of thiacyanine dyes in terms of the charge on each kind of molecule and with or without the formation of J-aggregates. First, for 0.5 µM 3,3′-diethyl thiacyanine, positively charged and not forming J-aggregates,[41] both a temporally-stable SERS spectrum and a temporally-fluctuated SERS spectrum were observed (data not shown). The former is almost the same as the Raman spectrum of the powder. Second, for 0.5 µM TC, negatively charged and not forming J-aggregates, a temporally-fluctuating SERS spectrum was exclusively observed (Figure 4a). Finally, for 0.5 µM TC-Cl_2, negatively charged and forming J-aggregates, the temporally-stable SERS spectrum, which is almost the same as the Raman spectrum of the J-aggregate, was exclusively observed (Figure 3b). The contrasting observations between 3,3′-diethyl thiacyanine and TC support the idea that the temporal fluctuation is enhanced by the negatively-charged functional groups, namely fluctuation in the Coulomb repulsion between two SO_3^- (3,3′-disulfopropyl) groups in TC and negatively-charged Ag surfaces. The contrasting observations between TC and TC-Cl_2 support the idea that the temporal fluctuation is suppressed by the formation of planar J-aggregates of TC-Cl_2.

Thermal or Photodegradation. Here we consider the possibility that the temporally-fluctuated SERS spectra are attributed to thermal or photodegradation of thiacyanine dyes from the following three viewpoints. First, Figures 4b and 4c show averaged temporally-fluctuated SERS spectra of a single Ag nanoaggregate adsorbed from a 0.5 µM TC solution and a 0.5 nM TC-Cl_2 solution excited at 458 nm for 360 and 132 s, respectively. No broad bands around 1580 and 1340 cm^{-1} ascribed to amorphous carbon[42,43] were observed in Figures 4b and 4c. Thus, the averaged temporally-fluctuating SERS spectra were quite different from a SERS spectrum of amorphous carbon due to degradation.[42,43]

Second, Figures 5a–5c show the excitation wavelength (458, 488, and 514 nm) dependence of the SERS and emission spectra of a single Ag nanoaggregate adsorbed from the same 0.5 µM TC-Cl_2 solution. Note that all the excitations were at the same power density (ca. 0.2 kW cm^{-2}). The ratio of Ag nanoaggregates that generated the SERS spectra to those that did not generate the SERS and the intensity of background emission decreased as the excitation wavelengths were tuned to those longer than the absorption maximum of TC-Cl_2 J-aggregate (464 nm). The SERS spectra excited at 458 and 488 nm in Figures 5a and 5b are similar to the Raman spectrum of J-aggregates in Figure 3a. On the other hand, Figure 5c shows that the SERS spectrum without background emission, which temporally fluctuated and similar to Figures 3c and 3e, was observed by excitation at 514 nm. This SERS spectrum is clearly different from the Raman spectrum of trisodium citrate dihydrate powder used as a reducer of Ag nitrate in the Lee–Meisel method in Figure 5d. Furthermore, it is confirmed that enough TC-Cl_2 molecules are adsorbed on the Ag nanoaggregate by the SERS spectra in Figures 5a and 5b. A SERS-active junction volume is too small to adsorb J-aggregate composed of many molecules. As usual, a section of J-aggregates could be just outside of the junction volume. Hence, the SERS signal excited at 458 nm may be mainly attributed to resonance Raman effect, and the SERS spectrum excited at 514 nm may not represent the entire J-aggregate. Thus, the SERS spectrum without background emission is not attributed to impurities but thiacyanine dyes.

Finally, a temporally-stable SERS spectrum of TC-Cl_2 adsorbed on a single Ag nanoaggregate in a NaCl solution (Figures 3b, 5a, and 5b) did not change into a temporally-fluctuating SERS spectrum under low excitation power density (ca. 0.2 $kW\,cm^{-2}$).[36] Therefore, the temporally-fluctuating SERS spectra without background emission are not attributed to thermal or photodegradation but to characteristics intrinsic to thiacyanine dyes.

Conclusion

We observed two types of SERS spectra: one is temporally-stable with background emission like that in Figures 3b, 5a, and 5b, the peaks of which are close to the Raman spectrum of J-aggregates (Figure 3a); the other is temporally-fluctuating without background emission like those in Figures 3c, 3e, 4a, and 5c. For a 0.5 μM TC-Cl_2 solution, the SERS spectrum is almost the same as the Raman spectrum of the J-aggregates and accompanied by background emission, which is fluorescence from the J-aggregates. We identified SERS spectra of TC-Cl_2 J-aggregates adsorbed on a single Ag nanoaggregate at higher dye concentrations. In a 0.5 nM TC-Cl_2 and a 0.5 μM TC solution, both of them likely exist in monomers and dimers, and thus SERS spectra without background emission were observed. No Raman spectra of a single Ag nanoaggregate adsorbed by a 0.5 nM TC-Cl_2 and a 0.5 μM TC solution were observed in the 1000–400 cm^{-1} region, where out-of-plane vibrational modes of J-aggregates are observed. The concentration-dependent SERS and background emission spectra were associated with whether J-aggregates are formed or not. Furthermore, SERS from a TC 0.5 μM solution showed temporal fluctuation likely due to thermally-driven Coulomb repulsion and attraction between the surface of Ag and TC monomers or dimers, both of which do not have planar adsorption geometry like TC-Cl_2 J-aggregates. Once J-aggregates of thiacyanine dyes were identified by SERS spectra, SERS spectroscopy might enable us to analyze step-by-step formation of J-aggregates at the single molecule level.

This study was partly supported by the Izumi Science and Technology Foundation (H19-J-24). The work was financially supported by "Open Research Center" project (Research Center for Near Infrared Spectroscopy) for private universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2001–2008. This work was also supported by KAKENHI (Grant-in-Aid for Scientific Research) on Priority Area "Strong Photon-Molecule Coupling Fields (No. 470, 20043032)" from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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