Raman Microprobe Spectrometer Installed in a Super-Conducting Magnet

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A Raman microprobe spectrometer that could be installed in the bore of a cryogen free super-conducting magnet (10 T) was designed and constructed for the investigation of the external magnetic field effect on the Raman spectra of molecular aggregates in solutions and at interfaces. The performance of the present instrument was demonstrated by measuring the magnetic field effect (0–10 T) on the resonance Raman spectra of diprotonated *meso*-tetra-(sulfonatophenyl)porphine aggregates in an acidic solution. The Raman shifts of the aggregates were not significantly influenced even in 10 T. However, the relative intensity of 1123 cm⁻¹ peak ($\nu(\text{C}_\alpha\text{-N})$) was interestingly enhanced about 20% under the magnetic fields higher than 2.5 T.

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Introduction

The external magnetic fields have been diversely utilized for spectroscopic investigations to characterize molecular structures and their functions, such as nuclear magnetic resonance, electron spin resonance, and magnetic circular dichroism. As for the Raman spectroscopy, Raman magnetic resonance of solid samples has been reported for the studies of spin-wave propagation (magnon excitation) and spin-flip scattering of ferromagnetic, anti-ferromagnetic, and ferrimagnetic crystals.^{1–4} However, the strong magnetic field effects on the Raman spectra of organic chromophores, biomolecules, or living cells have rarely been investigated.^{5,6} A Raman microprobe spectrometer has enabled Raman analysis of very small regions of *ca.* 1 $\mu\text{m} \times 1 \mu\text{m}$ for very tiny samples or heterogeneous samples.⁷ Recently, a cryogen-free super-conducting magnet has been developed, and a high magnetic field of 8–10 T has become usable for various kinds of researches.^{8–13} Based on these instrumental improvements, we have designed and constructed a high performance Raman microprobe spectrometer in a bore of a super-conducting magnet (10 T) optimized for the analysis of magnetic field effect on the Raman spectra of small regions in bulk solutions and at liquid/liquid interfaces.

The performance of this Raman spectrometer was examined by measuring the resonance Raman spectra of porphyrins in solutions. Synthetic water-soluble porphyrins have been watched with keen interest to investigate the functions and structures of their native porphyrin derivatives in biological and physiological states. Among them, *meso*-tetra(sulfonatophenyl)-porphine (TPPS) (Fig. 1) has been particularly employed for photodynamic therapy as a photosensitizer.^{14,15} Another characteristic of this and other porphyrins is the formation of micrometer-length rod-like J-aggregates in bulk solutions and at liquid/liquid interfaces, which has been confirmed with UV-Vis absorption spectroscopy, resonance Raman spectroscopy, and

atomic force microscopy.^{16–23} Also, it was reported by Boafma *et al.* that these diamagnetic molecular aggregates might be aligned with external magnetic fields.²⁴ These reports encouraged us to investigate the effects of high magnetic fields on the diamagnetic molecular aggregates in solutions and at liquid/liquid interfaces. In the present work, the magnetic field effects on the resonance Raman spectra of the diprotonated TPPS aggregates in solutions have been observed by using our newly constructed Raman microprobe spectrometer.

Experimental

A Raman microprobe spectrometer installed in a bore of the super-conducting magnet

Figure 2 illustrates a block diagram of a Raman microprobe spectrometer setup. The incident beam from an argon ion laser depolarized by a quarter-wave plate is introduced with an optical fiber (single mode, 10 μm in core diameter, 5 m in length) to the microprobe head in an open bore (10 cm in diameter and 95 cm in length) of a cryogen free super-conducting magnet (JMTD-10T100HH1, Japan Magnet Technology, Japan). Figure 3 shows a photograph of the

Fig. 1 A chemical structure of diprotonated TPPS monomer.

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Fig. 2 A schematic diagram of the Raman microprobe spectrometer.

Fig. 3 A photograph of the microprobe head (Mode I and Mode II) being installed in a bore of the super-conducting magnet. White lines indicate laser lines.

Raman microprobe head being installed in the bore. During a measurement, the microprobe head is completely inserted into the bore so as to be positioned at the maximum magnetic field. The wavelength of the laser is either 488.0 nm or 514.5 nm (GLG 3001 and GLG 3103, Showa Optronics Co., Ltd., Japan). In the microprobe head, the incident beam is collimated with an objective (20 $\times$). The polarization of the incident beam is controlled by a linear polarizer. The scattered light from a micro-cell is collected through a long working distance objective (20 $\times$, NA 0.40, LMPlanFI, Olympus Corporation). Then, the collected light is passed through a holographic notch filter (Notch-Plus, Kaiser Optical Systems Inc., Japan) and is transferred from the magnet bore using another optical fiber (single mode, 50 μ m in core diameter, 5 m in length) to a spectrograph (resolution = ± 2 cm^{-1} , Photon Design Co., Ltd., Japan) equipped with an EEV CCD detector (LN/CCD Detector, Princeton Instruments) cooled to 153.15 K with liquid nitrogen. Both the position of a micro-sample cell on an X, Y, and Z stage and the polarization of the incident beam are controllable from the outside of the magnet bore by mechanical gears. A sample on the stage is observable through the other CCD camera. The focal point of the objective is located inside a cylindrical space (40 mm in diameter and 10 mm in length), in which the uniformity of the magnetic fields is guaranteed better than 99.99%.

The new instrument has 5 different modes for Raman measurement geometries available. Three variations in measurement geometries are shown in Fig. 4, in which the scattered light is collected through the objective from a direction perpendicular to the sample solution surface. The first

Fig. 4 Magnetic field geometry perpendicular to the observation axis of an objective; (A) Mode I, and (B) Mode II and Mode III.

Fig. 5 Magnetic field geometry perpendicular to the observation axis of an objective; (A) Mode IV, and (B) Mode V.

one (Mode I) of the three variations is the 180° geometry (Fig. 4(A)), in which the incident beam irradiates the sample vertically and the scattered light is collected by the same objective. The second (Mode II) and third (Mode III) ones are the oblique irradiation modes set by the selection of the X and Y directions, respectively, for the incident angle of 65°–75° adjusted by a variable angle mirror (Fig. 4(B)). These are suitable for the total internal reflection measurements at the liquid/liquid interfaces and the liquid/solid interfaces. In these three geometries, the magnetic field is applied parallel to the interface of the sample solution. The fourth (Mode IV) and fifth (Mode V) geometries refer to the choice of 180° geometry (Fig. 5(A)) and 65°–75° geometry (Fig. 5(B)) for the incident beam, respectively; in these modes the scattered light is collected from a direction perpendicular to the solution surface. In both situations, the magnetic field is applied perpendicularly to the surface or interface of the sample solution.

Measurements of absorption and resonance Raman spectra

The water-soluble porphyrin, *meso*-tetra(sulfonatophenyl)-

Fig. 6 Absorption spectral changes due to the aggregation of diprotonated TPPS in 0.2 M HCl.

porphine (TPPS), was purchased from Porphyrin Products, Inc., and used without further purification. Solutions were prepared with using distilled and deionized water by a Milli-Q system. The pH value of aqueous solutions was adjusted by the addition of 0.2 M HCl. The solutions were kept at ambient temperature and in a dark location prior to spectral measurements for 12 h in absorption spectral measurements and for 48 h in Raman spectral measurements to establish the porphyrin aggregate equilibrium.

Resonance Raman scattering of diprotonated TPPS aggregate in aqueous solutions was excited at 488 nm with the laser power of 0.1 mW on the focal plane of the objective. The spectra were taken under the condition of 5 s exposure time of CCD detector and 5 accumulations. The optical observation geometry was 180° backscattering, and the directions of the incident beam and the magnetic fields were perpendicular to each other (Mode I). The polarization of the incident beam was adjusted to be parallel to the direction of the magnetic fields. The sample solutions for Raman spectral measurements were sealed in a square fused silica capillary tube with a sectional area of 100 $\mu\text{m} \times 100 \mu\text{m}$. Absorption spectra were measured by a UV/VIS/NIR spectrophotometer (V-570, JASCO, Japan) using an ordinary 10 mm quartz cell. All spectral measurements were conducted at ambient temperature.

Results and Discussion

It was reported that TPPS diprotonated molecules formed micrometer-sized aggregates at rather high analytical concentrations and in a low pH solution.^{17,19,23} At first, we confirmed the aggregation of TPPS molecules in acidic solutions by absorption spectroscopy. Figure 6 shows absorption spectra of diprotonated TPPS solutions in 0.2 M HCl at different TPPS concentrations from 1.16×10^{-6} M to 1.39×10^{-5} M. Since green precipitates or aggregates were observed after 12 h in the higher concentrations over 2×10^{-5} M, the absorption spectra were examined only in the lower concentrations. The spectrum of 1.16×10^{-6} M TPPS solution showed only a monomer peak at 435 nm. With an increase in the concentration of TPPS, the 435 nm peak was shifted to 433.5 nm, and two new peaks appeared at 425 nm and at 491 nm. An isosbestic point at 450 nm clearly indicated the mass-balanced reaction at pH 0.8 between diprotonated TPPS monomers and their aggregates.

The aggregate number n and the aggregate equilibrium

Fig. 7 Raman spectra of 5.0×10^{-5} M TPPS in 0.2 M HCl solution measured under the magnetic fields (0, 5, and 10 T) after 48 h equilibration outside the bore.

constant K were calculated from the following equations:²⁵

$$\log c_0 \left(1 - \frac{\varepsilon}{\varepsilon_m} \right) = \log(CK) + n \log c_0 \left(\frac{\varepsilon}{\varepsilon_m} - \frac{\varepsilon_n}{n\varepsilon_m} \right) \quad (1)$$

$$C = \frac{1}{n^n \left(n - \frac{\varepsilon_n}{\varepsilon_m} \right)^{n-1}} \quad (2)$$

$$\varepsilon = \frac{1}{n} x \varepsilon_n + (1 - x) \varepsilon_m \quad (3)$$

where c_0 is the analytical concentration of diprotonated TPPS, $(1 - x)$ is the mol fraction of the diprotonated monomer of TPPS, ε is the apparent molar absorptivity at 435 nm, and ε_m and ε_n are the molar absorptivities of monomer and aggregate at 435 nm, respectively. From the linear fitting of experimental values in Eq. (1), the aggregate number and the aggregation equilibrium constant were determined as $n = 3$ and $K = 7.33 \times 10^{13}$.

Next, the magnetic field effects on the resonance Raman spectra of diprotonated TPPS aggregates were investigated under different magnetic field strengths. The TPPS concentration for resonance Raman spectral measurements was adjusted to 5.00×10^{-5} M. As the growth of the aggregate size, which was observed with a microscope, improved the signal to noise ratio of the Raman spectra, the sample solution was kept for 48 h. Figure 7 shows the resonance Raman spectra of the TPPS aggregates (5×10^{-5} M) in 0.2 M HCl solutions under 0, 5, and 10 T. From Fig. 7, any remarkable changes of the Raman shifts were not observed, which were mostly shifted by less than 1 cm^{-1} even in 10 T. This indicated that even 10 T magnetic fields did not induce the energy level shifts of normal vibrational modes larger than the spectrograph resolution of $\pm 2 \text{ cm}^{-1}$. Figure 7 also shows the decline of the absolute intensity of Raman signal from 0 T to 10 T. This might be caused by the heterogeneous distribution of diprotonated TPPS micrometer-sized aggregates in a capillary cell or by the photo-thermal decomposition of the aggregates.

The magnetic field effect was then discussed based on the relative intensities of principal Raman peaks to a 1231 cm^{-1} ($\nu(\text{C}_m-\Phi)$) peak intensity. Figure 7 shows the relative peak intensities normalized to 0 T at different magnetic field strengths. The relative intensity corresponding to 1123 cm^{-1}

Fig. 8 Magnetic field effect on the relative Raman intensity ratios of principal peaks to the intensity of 1231 cm^{-1} peak normalized at 0 T.

($\nu(\text{C}_a\text{-N})$) peak was gradually enhanced by about 20% from 0 T to 2.5 T and was saturated above 2.5 T. The relative intensities for other shifts of 697 cm^{-1} (not defined), 986 cm^{-1} (pyrrole breathing), 1016 cm^{-1} (pyrroline ring breathing), 1474 cm^{-1} ($\nu(\text{C}_b\text{-C}_b)$), 1534 cm^{-1} ($\nu(\text{C}_b\text{-C}_b)$), and 1561 cm^{-1} ($\nu_{as}(\text{C}_a\text{-C}_m)$) were not affected by the magnetic fields even in 10 T. The results of resonance Raman relative intensities show that the magnetic fields might magnetically induce the deformation of molecular transition polarizability tensors at the inner macrocycle of diprotonated TPPS. The Raman shifts of spectra were assigned according to Ref. 26.

The magnetic field effect on the relative intensities of diprotonated TPPS aggregates (Fig. 8) is very interesting, but the reason for it is still ambiguous at the present stage. However, some insights have been reported, though the direct relevance with our observations is not yet settled. For example, the magnetic field-inducing orientation of phthalocyanine aggregates in chloroform was found by polarized absorption spectroscopy.²⁴ The spectral results showed that the aggregates were oriented parallel to the applied field direction with increasing of the magnetic fields, and the orientation was saturated at 3 T. Barron *et al.* reported the magnetic resonance Raman optical activity spectra of ferrocyclochrome *c* excited at 514.5 nm under the magnetic field strength of 0.7 T. They indicated that the peak around 1123 cm^{-1} was an anomalously polarized band.⁶ The magnetic field effects on the lifetime of the biradicals consisting of benzophenone-acetophenone ketyl radicals²⁷ and on the decay rate constant of triplet biradicals in zinc-tetraphenylporphyrin-fullerene linked compound ($\text{ZnP}(8)\text{C}_{60}$)²⁸ were also saturated before 2.5 T. The feature of the magnetic field effect observed in the present study, which showed saturation in 2.5 T, was similar to these previous reports. We are conducting theoretical analyses and systematic experiments to elucidate the mechanism of magnetic field effects on the resonance Raman spectra of the diprotonated TPPS aggregates, other molecular aggregates, and other biomolecules. For these purposes, the Raman microprobe spectrometer newly constructed in the present study will be a powerful tool.

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