Anti-Stokes fluorescence from chlorophyll a

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Abstract. Anti-Stokes fluorescence from chlorophyll a molecules dispersed in diethyl ether has been observed. From the excitation power dependence of the fluorescence spectrum, it is concluded that the anti-Stokes fluorescence appears via the linear optical process. To consider the relationship between the observed spectral shape and vibrational modes, the model spectral density has been determined based on the Raman spectrum and real-time coherent vibrational oscillation signals. The absorption spectrum observed in the experiment was well reproduced by the calculation, indicating the validity of the model spectral density. The involvement of the low-frequency vibrational modes in the anti-Stokes fluorescence process is discussed.

1. Introduction
Chlorophylls (Chl) and bacteriochlorophylls (BChl) play important roles in the initial steps of photosynthesis [1]. For example, BChl a is known as a key pigment of light-harvesting antennas of purple bacteria. Reflecting the periodic one-dimensional arrangement of BChl a, the excitation energy transfer between molecules following light harvest is well explained by introducing the concept of an exciton. Further, in photosystem II of higher plants, coherent propagation of excitons between Chl a molecules has been observed. It is therefore interesting to elucidate the relationship between the electronic structure and optical responses of (B)Chl, which covers a wide spectral range of sun light.

Recently, clear anti-Stokes fluorescence from chloroplasts in Parachlorella kessleri cells and in leaves of Zea mays has been observed at room temperature [2,3], while the underlying mechanism, e.g., the influence and necessity of the protein environment surrounding pigments for the anti-Stokes fluorescence process, is still not fully understood. In the present study, we investigated the fluorescence spectra from Chl a molecules dispersed in diethyl ether over a wide spectral range. The relationship between the anti-Stokes fluorescence and molecular vibration modes is discussed.

2. Experimental
Chl a was purchased from Chlorophyll Research Institute Ltd. and used without further purification. As typically used [1], diethyl ether was employed as the solvent to prevent specimen degradation. The conventional absorption spectrum was recorded at room temperature on a commercial spectrophotometer (Shimadzu, UV-1850). The excitation light source used for the conventional fluorescence measurement was a He-Ne laser, while that for the anti-Stokes fluorescence measurement was a cw Ti:sapphire laser (Spectral Physics, 3900S) pumped by a 5-W laser diode (Spectral Physics,
The spectra were collected with a 30-cm single monochromator (Zolix, Z-300) and a cooled CCD camera (Mitty-CCD, H101411107-DS).

3. Results and discussion

Figure 1 shows the absorption and fluorescence spectra of Chl a dispersed in diethyl ether. The absorption peaks observed at 15100 and 17300 cm$^{-1}$ are the so-called $Q_y$ and $Q_x$ bands, respectively. The small absorption peak at 16300 cm$^{-1}$ originates from the quantization of vibrational levels of $Q_y$, i.e., the peak reflects the transition from the electronic ground state to the first vibrational excited level coupled with the electronic $Q_y$ band. We note that the symbols $(m,n)$ in Fig. 1 indicate the transition between the $m$-th vibrational level in the electronic ground state and the $n$-th vibrational level in the excited state. The dash-dotted curve in Fig. 1 shows the conventional fluorescence spectrum, where the excitation was with a He-Ne laser at 15800 cm$^{-1}$, which is slightly below the $Q_x$ band. The main peak of the fluorescence spectrum at 14900 cm$^{-1}$ is slightly redshifted compared to the 0-0 transition peak observed in the absorption spectrum due to the solvent reorganization. The fluorescence spectrum forms the mirror image of the absorption spectrum: the relative fluorescence intensity of the $(0,0)$ and $(0,1)$ peaks and the energy separation of these peaks are very close to those of the $(0,0)$ and $(0,1)$ peaks in the absorption spectrum. By contrast, the fluorescence spectrum upon the excitation below the $(1,0)$ peak shows a significantly different shape, as shown by the thick solid curve in Fig. 1. Namely, another peak marked as X appears between the $(0,0)$ and $(1,0)$ peaks observed in the conventional fluorescence spectrum. In the following part of the present paper, we explore the possible mechanism of the significant change in the spectral shape.

The anti-Stokes fluorescence was measured at various excitation powers. The excitation power was changed over 2 orders of magnitude. The anti-Stokes fluorescence intensity also increases with the increase in excitation power. The relative intensities of the peaks at 14300 and 14900 cm$^{-1}$ were almost constant within the excitation power range measured in the present study: these peaks linearly increase with the excitation power. This fact indicates that the anti-Stokes fluorescence originates from a one-photon process.

Figure 2 shows a series of fluorescence spectra measured at various excitation energies. It should be noted that each spectrum is magnified and that the baseline is vertically shifted for clarity. The top curve in Fig. 2a was measured upon the excitation at 13300 cm$^{-1}$, which is approximately 1600 cm$^{-1}$ below the 0-0 transition energy of the $Q_y$ band. The $(0,0)$ peak at 14900 cm$^{-1}$ is approximately twofold stronger than the peak X at 14300 cm$^{-1}$. With the decrease in excitation energy, the fluorescence intensity decreases. In addition, the spectral shape changes considerably: when the excitation is set to approximately 13000 cm$^{-1}$, the intensity of the fluorescence peak X is significantly enhanced. The $(0,0)$ peak becomes relatively stronger again when excited at much lower energies. When the
The excitation energy is detuned from 13000 cm$^{-1}$, the relative intensity monotonically decreases, and the peak X ultimately becomes very weak.

To investigate the contribution of vibrational oscillations to the anti-Stokes fluorescence process from Chl$\text{a}$, a model spectral density $\rho(\omega)$ was determined from the Raman spectra and real-time coherent vibrational oscillations reported in previous studies [4–6]. Reflecting the complicated structure of Chl$\text{a}$, it is well-known that many Raman peaks appear. In the present study, only the outstanding peaks are included in the model spectral density, as shown in Fig. 3a. In addition, the influence from the solvent is included by introducing a broad low-frequency peak into the spectral density. In the present study, the line shape of each phonon mode is simply approximated with a Lorentzian function [7].

The steady-state absorption spectrum $\sigma_A(\omega)$ is then calculated using the line broadening function $g(t)$ [8,9], i.e.,

$$\sigma_A(\omega) \propto \text{Re} \int_0^\infty dt \exp\{i(\omega - \omega_{eg})t - g(t)\}. \tag{1}$$

The cut-off frequency is set to be 1800 cm$^{-1}$ when the numerical integrations are performed. The absorption spectrum of Chl$\text{a}$ calculated in this way is shown by the dotted curve in Fig. 3b. The experimentally observed absorption spectrum (solid curve in Fig. 3b) is well reproduced, indicating the validity of the spectral density constructed here. The separation between the 0-0 and 0-1 vibrational transitions is approximately 1200 cm$^{-1}$, which mainly reflects the distribution of the vibrational modes of Chl$\text{a}$. Namely, as seen in Fig. 3a, most of the vibrational modes are in the frequency range of 800 cm$^{-1}$ to 1600 cm$^{-1}$, which determines the energy separation between the (0,0) and (0,1) peaks of the absorption spectrum or between the (0,0) and (1,0) peaks of the ordinary
fluorescence spectrum upon the excitation above the Q_y band. It should be mentioned that the vibrational peaks (0,0) and (0,1) are clearly separated compared to those observed in BChl a [9], which indicates that the interaction between the Chl a molecules and the surrounding solvent is weaker than that between BChl a and the solvent.

In the case of the excitation on the anti-Stokes side of the 0-0 transition energy, the molecule seems to dominantly couple with rather low frequency modes of approximately 400 cm\(^{-1}\). The detailed mechanism of this phenomenon is still unclear, but it has been shown that the coupling strength of the vibrational modes depends significantly on the excitation energy [5,6]. The unusual spectral shape observed in the anti-Stokes fluorescence could suggest that the fluorescence appears before the thermal equilibrium in the Q_y band is attained. It should also be noted that no significant change in the spectral shape around the peak X was observed at sample concentrations between 15 and 90 μM. Therefore, we consider that the aggregation of Chl a molecules is a less plausible origin of the anti-Stokes fluorescence. To prove these hypotheses, it is necessary to measure time-resolved fluorescence over a wide excitation energy range as well as the solvent and temperature dependences, but these are left as the subjects for future studies.

To summarize, we have observed the anti-Stokes fluorescence from Chl a molecules in an organic solvent. This fact indicates that the anti-Stokes fluorescence can emerge without any assistance from a protein environment. The model spectral density that leads to the spectral shapes of conventional absorption and fluorescence spectra has been determined. It is plausible that among the many vibrational modes, the low-frequency modes of approximately 400 cm\(^{-1}\) have a significant influence on the spectral shape of the anti-Stokes fluorescence.

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