Estimation of Quantum Yields of Weak Fluorescence from Eosin Y Dimers Formed in Aqueous Solutions

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We studied weak fluorescence from the dimer of eosin Y (EY) in aqueous solution. We used a newly developed ultrathin optical cell with a thickness on the order of microns to several hundred microns to successfully measure the fluorescence spectra of highly concentrated aqueous solutions of EY without artifacts caused by reabsorption of fluorescence. The spectra we obtained were similar to the fluorescence spectrum of the EY monomer; almost no fluorescence from the EY dimer was observed. By careful comparison of the spectra of solutions at low and high concentrations of EY, we succeeded in extracting the fluorescence spectrum of the EY dimer. The fluorescence quantum yield of the EY dimer was estimated to be 0.005.

Introduction

Fluorescence behavior (i.e., spectrum, lifetime, and quantum yield of fluorescence) is known to be affected by associations of molecules. The numerous studies of these effects that have been carried out constitute one of the most important fields of photochemistry. However, many issues related to these effects have yet to be resolved. It has often been observed, for example, that fluorescence intensity decreases as the concentration of molecules in solution increases.[1] This phenomenon is widely known as concentration quenching, but the details of the mechanism are still being debated. Studies of excimers and exciplexes[1] are important for understanding the fluorescence caused by molecular associations. In the case of these chemical species, characteristic fluorescence behavior that is significantly different from that of the constituent molecules can be observed after molecular association in the excited state. Recently, attention has been paid to the fact that non-emissive molecules under isolated conditions emit strong fluorescence after molecular aggregation. Actually, this behavior has been studied in the context of aggregation-induced emission (AIE).[2] Concepts obtained through studies of the influence of molecular associations on fluorescence behavior are therefore important not only for fundamental photochemistry but also for applications such as fluorescence probes.[3,4] As mentioned above, the effect of aggregation on fluorescence behavior is an important issue for various fields of photochemistry and thus detail mechanism should be understood from basic science point of view.

Because aggregates are formed under highly concentrated conditions in solutions, a highly concentrated sample is required for studies of fluorescence from aggregates. Generally, in samples with an absorbance larger than 0.1, fluorescence photons emitted from the molecules in the solution are absorbed again by the sample solution, a phenomenon characterized as the reabsorption effect of fluorescence.[1] Because of this reabsorption effect, the short-wavelength side of fluorescence spectra is effectively attenuated. In addition, fluorescence lifetimes (τ_F) appear to become long, and fluorescence quantum yields (Φ_F) appear to decrease. Note that the experimental results affected by the reabsorption effect are not suitable for discussion of detail mechanism of fluorescence properties. To elucidate details of the mechanisms responsible for the fluorescence emitted by aggregates formed in highly concentrated solutions, it is therefore very important to eliminate reabsorption artifacts on apparent fluorescence behavior. Several attempts have been carried out to eliminate reabsorption artifacts. For example, data have been analyzed based on a phenomenological model,[5] and measurements have been made using very thin optical cells.[6-8] Use of these methods effectively corrects the shape of the fluorescence spectra and fluorescence lifetime. However, it is difficult to evaluate Φ_F based on these techniques because the shape of the cells is not appropriate.

Dimerization of xanthene dyes in highly concentrated aqueous solutions has long been known as a prototype for concentration quenching caused by dimer formation.[9,10] When these dyes are dissolved in water at high concentration, the fluorescence intensity decreases dramatically. From analysis of the absorption spectra, it is clear that dimers are formed in the ground state, and suppression of fluorescence intensity is caused by static quenching by the non-fluorescent dimer. Although several attempts have been carried out to observe fluorescence spectra of the dimer,[11,12] obtained results are not necessarily reliable because of low efficiency of dimer

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fluorescence and reabsorption artifacts. Various hypotheses have been posed to explain the non-fluorescent properties of such dimers. Several mechanisms have been proposed, including efficient intersystem crossing (ISC) to a triplet excited state, rapid dissociation of an excited dimer to two ground state monomers, and rapid internal conversion (IC) to the ground state of the dimer. Although details of the mechanisms are expected to differ among dyes, for rhodamine B (RhB), fluorescence quenching of the dimer has been attributed to relaxation of the dimer excited state to the ground state within 100 ps.[13] In a recent transient absorption study with rhodamine 800, dissociation into excited monomers has been noted in addition to the fact that the lifetime is only 3 ps.[14] In addition to such real time measurements of relaxation, photoacoustic measurements of the heat generated by the relaxation of excited dimers has also been performed.[15] According to that study, IC is the dominant relaxation process, and ISC and dissociation of dimer excited states are not important for RhB and eosin Y (EY) dimers in aqueous solutions. It is interesting to note that rapid IC occurs for dimers but not for monomers. Such observations are important to advance understanding of non-radiative processes in excited state molecules including aggregates and to further development in various applied fields based on the florescence properties of aggregates.

As mentioned above, it has been known for a long time that dimers of xanthene dyes are not fluorescent, and it has been argued that rapid IC is likely the mechanism responsible for this behavior. However, in reality, no reliable results have been reported for the fluorescence spectra of the dimers and their quantum yields, whereas these results are very important for further understanding of the relaxation processes of the dimer in the excited state. This is because effective elimination of reabsorption effects in a fluorescence measurement with a highly concentrated solution is very difficult. Furthermore, even in highly concentrated solutions, highly fluorescent monomers coexist with dimers, and that intense fluorescence affects the measurement of fluorescence of a dimer with a low Φ_F.

In this study, we used an aqueous solution of EY to examine the emission spectra and fluorescence yields of EY dimers. EY is a xanthene dye that is widely used in various fields such as cosmetics because of its vivid orange color. Like many xanthene dyes, it has long been known to form dimers in aqueous solutions.[16-18] Dimers of EY can therefore be considered suitable examples of xanthene dye dimers. As mentioned above, the effect of reabsorption is a serious problem for detail discussion of fluorescence properties. To overcome this problem, we developed a thin optical cell with a thickness on the order of microns to several hundred microns. Using the cell, we can effectively eliminate the effect of reabsorption of fluorescence. Furthermore, by using a multichannel detector, we were able to measure the fluorescence spectra with a high signal-to-noise ratio and succeeded in extracting the fluorescence spectrum of the EY dimer, although it was small compared to the fluorescence spectrum of the dominant monomer, even in a highly concentrated solution. Based on these reliable experimental results, we could estimate the fluorescence quantum yield of EY dimer to be 0.005.

Experimental

Eosin Y (Nacalai Tesque) was used without further purification. To enhance dimerization of EY, an aqueous solution of 1 M NaCl was used as the solvent instead of pure water. Fluorescence spectra and decay profiles of a 1 μM solution of EY were measured with a time-resolved luminescence spectrometer based on a streak camera (Hamamatsu, C4334). Samples were excited by the second harmonic (400 nm) of a Ti:sapphire laser (Spectra-Physics, Tsunami). The repetition rate of the oscillator (80 MHz) was reduced to 8 MHz with a pulse selector (Spectra-Physics, Model 3980,). An absolute photoluminescence quantum yield spectrometer (Hamamatsu, Model C11347) was used to carry out the Φ_F measurements of the 1 μM solution of EY.

For solutions containing higher EY concentrations, absorption and fluorescence spectra were difficult to measure because of the artifacts caused by the high absorbance and reabsorption on the fluorescence. We therefore examined the absorption and fluorescence spectra using a specially designed cell with a short optical path length and a thickness on the order of microns to several hundred microns. A small amount of sample solution was placed between two 140 × 140 mm^2 square quartz plates. Pressure was then applied with two aluminum plates to both sides of the quartz plates to create a very thin liquid film between them. A hole with a diameter of 30 mm at the center of each aluminum plate made it possible to measure the absorption and fluorescence spectra of the thin liquid films through the holes. The optical path length of the sample solution could be adjusted by inserting a spacer between the two quartz plates. The optical path length of the thin liquid films was determined directly by measuring the interference of the transmitted light. To measure absorption spectra, white light from a halogen lamp (Ocean Optics, LS-1-CAL) was transmitted through the liquid film and was detected with a CCD spectrometer (B&W Tek, BRC 112 E-U) through an optical fiber. For fluorescence measurements, excitation light at 480 nm was obtained with a Xe lamp (Hamamatsu, C2577) after passing through a band pass filter (Asahi Spectra, PB0051) and an interference filter (Sigmakoki, VPFHT-25C-4880). For weak fluorescence measurements from the thin liquid films, a sensitive cooling CCD detector (Princeton Instruments, PIXIS 250) equipped with a monochrometer (Acton, Spectra Pro150) was used. To eliminate reabsorption effects on fluorescence, fluorescence measurements were performed by adjusting the optical path length so that the maximum absorbance of the EY (517 nm) did not exceed 0.1.

Transient absorption measurements were carried out with a Nd^{3+}:YAG laser. The second harmonic (532 nm) of the laser was used for excitation, and a Xe lamp (Hamamatsu, C7535) was used as the probe light source. The repetition rate of the laser was 10 Hz. The light transmitted through a sample in a cuvette with an optical path length of 2 mm was detected with

a Si photodiode (Thorlabs, DET10C/M) after being dispersed with a monochromator (Spectra Products, CM110). The photocurrent from the detector was amplified with an amplifier (Femto, DHPVA-100), and signals were processed with a digital oscilloscope (Tektronix, TDS380) and analyzed with a computer. Because the DC offset of the photocurrent from the detector was subtracted using the AC-coupled mode of the amplifier, small absorbance changes ($<10^{-4}$) could be detected. The time resolution of the system was about 20 ns.

Results and discussion

Absorption and fluorescence spectra of EY monomer

Evaluation of the fluorescence behavior of the EY dimer required a knowledge of the basic properties of the EY monomer. We therefore investigated the effect of the NaCl additions on the photo-physical properties of the EY solutions because 1 M NaCl aqueous solutions were used to enhance dimerization in this study. The absence of dimers in dilute solutions was confirmed by examining the absorption spectra. The blue line in Fig. 1a shows the absorption spectrum of a 0.01 mM solution of EY in a 1 M aqueous solution of NaCl. The spectrum is very similar to that of a 1 μM solution of EY (data not shown). This similarity clearly indicates that dimerization can be ignored in this concentration range. We also observed that there was no significant difference in the spectral shapes of these absorption spectra versus aqueous solutions without NaCl. These results indicate that no significant effects on the electronic state of the EY monomer were associated with the addition of NaCl.

To study the fluorescence properties of the EY monomer, we placed a 1 μM solution of EY in a square quartz cell with an optical path length of 1 cm. The absorbance at the peak was 0.08. The effect of reabsorption could therefore be ignored in the fluorescence measurements. The spectrum, quantum yield, and fluorescence lifetime of this 1 μM solution were then measured, and the influence of NaCl was investigated. The fluorescence spectrum of EY was very similar in a 1 M NaCl aqueous solution and in an aqueous solution without NaCl. In addition, the fluorescence quantum yield was determined to be 0.23, which is close to the value reported in an aqueous solution (Φ_F = 0.20).[17] The fluorescence lifetime was determined to be 1.1 ns, which is also close to the value reported in an aqueous solution (τ_F = 1.425 ± 0.140 ns).[17] These experimental results clearly indicate that addition of NaCl to the solvent did not affect the relaxation processes of the EY monomer in the excited state. With the values of the fluorescence quantum yield Φ_F^M and fluorescence lifetime τ_F^M of the EY monomer obtained in this study, we were able to determine that the radiative lifetime τ_R^M of the EY monomer was 5.0 ns using the equation $\tau_R^M = \tau_F^M / \Phi_F^M$.

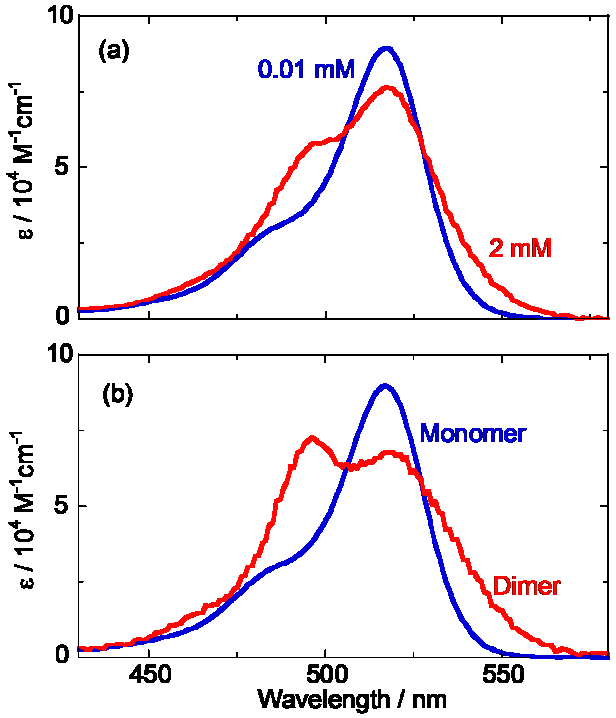

Fig. 1 Absorption spectra of EY at 0.01 mM and 2 mM in NaCl (1 M) aqueous solution (a). Monomer and dimer component of the absorption spectra (b).

Absorption spectra of EY dimer

Absorption spectra were used to study dimerization of EY in highly concentrated solutions. Figure 1a shows the absorption spectra of 2 mM and 0.01 mM EY solutions (red and blue lines, respectively) in a 1 M NaCl aqueous solution. It is apparent that the absorption spectra becomes broader with increasing EY concentration. This spectral change is known to be caused by formation of EY dimers. It should be pointed out that even in a 2 mM (i.e., highly concentrated) EY solution, EY monomers coexist with EY dimers. The mole fraction of the EY dimer, X_D, is therefore needed for quantitative analysis.

Absorption spectra of EY at a series of concentrations (0.01 mM, 0.05 mM, 0.1 mM, 1 mM, 2 mM, and 5 mM) were measured to obtain absorption spectra and X_D values (Fig. S1). It is noted that all spectra shown in Fig. S1 could be reproduced by using absorption spectra of EY monomer and EY dimer. In addition, X_D should be explained by following relation. The molar extinction coefficient $\varepsilon(\lambda)$ of the sample solution at a given concentration and wavelength can be expressed as a function of the molar extinction coefficient of the monomer $\varepsilon_M(\lambda)$ and of the dimer $\varepsilon_D(\lambda)$ and the mole fraction, X_M, of the EY monomer as follows[18]:

$$\varepsilon(\lambda) = \varepsilon_M(\lambda)X_M + \varepsilon_D(\lambda)(1 - X_M) \quad (1)$$

The equilibrium constant K_D for the dimerization (M + M ↔ D) can be expressed as follows[18]:

$$K_D = \frac{(1-X_M)}{2X_M^2 C} \quad (2)$$

where C is the nominal dye concentration. From eqs. (1) and (2), it follows that

$$2K_D C = (1 - \frac{\varepsilon(\lambda)-\varepsilon_D(\lambda)}{\varepsilon_M(\lambda)-\varepsilon_D(\lambda)})/((\frac{\varepsilon(\lambda)-\varepsilon_D(\lambda)}{\varepsilon_M(\lambda)-\varepsilon_D(\lambda)})^2) \quad (3)$$

K_D and $\varepsilon_D(\lambda)$ were evaluated to reproduce the observed spectra properly. After trial and error, we obtained molar fractions as a function of EY concentration as shown in Fig. S2. The obtained value of K_D (K_D = 900) is larger than the value of K_D reported for EY in aqueous solutions of 0.01 M KCl (K_D = 110 at a pH of 12 and K_D = 115 ± 3 at a pH of 12).[16,18] This difference is probably due to the use of NaCl at high concentration (1 M), because NaCl promotes dimerization. The absorption spectrum of the EY dimer can be extracted from the observed spectra using the values of K_D and $\varepsilon_D(\lambda)$. Figure 1b shows the absorption spectra of the dimer (red line) and monomer (blue line). Two absorption peaks at 497 nm and 522 nm appear for the EY dimer. In contrast, the EY monomer has only one absorption peak. Such characteristic changes of the absorption bands at higher EY concentrations are known to be due to dimerization.[16-18] Actually, the ratio of the intensity of the longer-wavelength peak to the intensity of the shorter-wavelength peak in the present results was slightly higher than previously reported. The reason is unclear. One possible explanation is the influence of NaCl, which promotes dimerization.

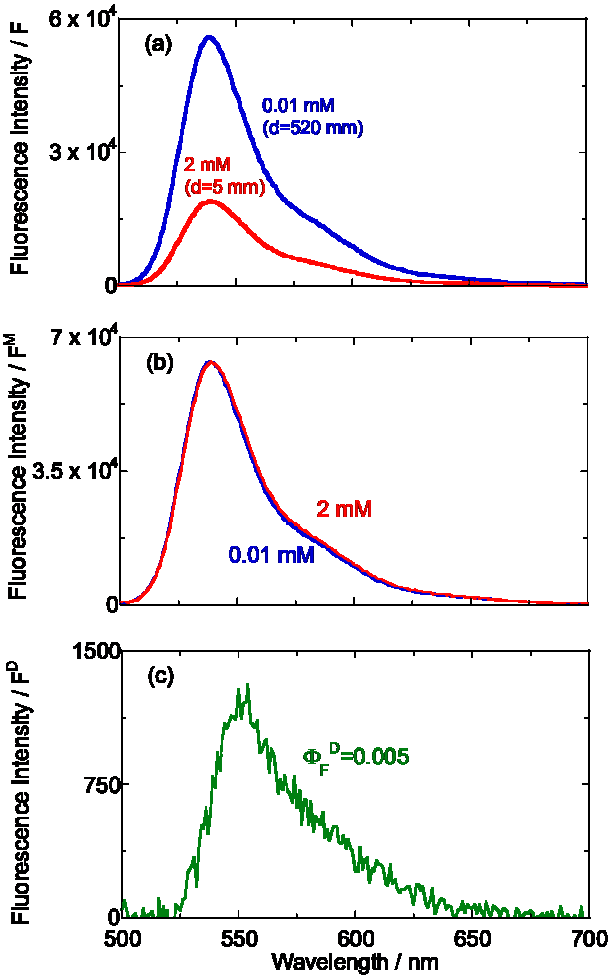

Fig. 2 Fluorescence spectra of EY at 0.01 mM and 2 mM in NaCl (1 M) aqueous solution normalized by the absorption fraction of the sample solution (a) and by the absorption fraction of the monomer (b). Difference spectrum between the fluorescence spectrum of a 2 mM solution and that of a 0.01 mM solution normalized at 520 nm (c).

According to the widely accepted model for the absorption spectra of dimers,[4] the relative ratio of the two absorption peaks of the dimer reflects the relative orientation of the two molecules in the dimer. Based on this reasoning, the absorption spectrum for the dimer obtained in the present study shows that the two EY molecules are not parallel to each other. Instead, they are oriented at an oblique angle. For such dimer, fluorescence emission occurs from the lower excited state after rapid internal conversion (IC) from the higher excited state. It should be noted that this fluorescence transition is an optically allowed transition because of an oblique structure. The absorption strength is known to be proportional to the degree to which a transition is optically allowed, and therefore the radiative lifetime (τ_R^D) of the EY dimer can be evaluated based on an analysis of the absorption spectra. The absorption spectra in Fig. 1b show the molar absorption coefficients plotted against wavelength. There are two optical transitions in the absorption spectrum of the EY dimer. The area of the absorption spectrum associated with the transition to the lowest excited state of the EY dimer is about half that of the EY monomer transition. Accordingly, the radiative lifetime of the EY dimer can be roughly estimated to be twice (τ_R^D = 10 ns) that of the monomer (τ_R^M = 5 ns).

Fluorescence spectra of the EY dimer

The fluorescence spectrum of 2 mM EY in a 1 M NaCl aqueous solution was measured carefully. The fluorescence properties of highly concentrated solutions are influenced by the effects of fluorescence reabsorption (vide supra), and therefore it is important to eliminate this artifact. It is known that the reabsorption effect can be efficiently suppressed when the maximum absorbance of the sample is less than 0.1. To realize this condition, we developed an ultrathin optical cell with a thickness on the order of microns to several hundred microns for fluorescence measurements of highly concentrated solutions. We measured the fluorescence of samples with EY concentrations of 0.01 mM and 2 mM. Only the monomer was present in the dilute solution (X_M = 0.99), and the monomer and dimer coexisted in the concentrated solution (X_M = 0.42).The optical path lengths used in the measurements were 520 μm and 5 μm for the 0.01 mM and 2 mM solutions, respectively. The analogous absorbances at the peak wavelengths were 0.046 and 0.045, respectively. Thus, the reabsorption effect was effectively suppressed.

The recorded fluorescence intensity is proportional to the product of the absorption fraction of excitation light (F), the fluorescence quantum yield (Φ_F), and the fluorescence collection efficiency (E) in the measurement. The value of F can

be obtained from the absorbance (*A*) obtained from absorption spectroscopic measurements using the equation $F = (1 - 10^{-A})$. It is reasonable that the value of *E* to be the same for both measurements because the fluorescence measurements were performed with the same optical geometry in this study. Accordingly, the recorded fluorescence spectra were normalized by *F* (Fig. 2). The difference in the signal intensity of the two spectra in Fig. 2a is an indication of the difference of Φ_F values. The clearly lower Φ_F of the concentrated solution versus the dilute solution is an indication of concentration quenching.

Because there were no dimers in the dilute solution, the fluorescence quantum yield of the dilute solution, Φ_F^{dil}, was the same as the Φ_F^M obtained with the 1 μM solution (Φ_F^{dil} = 0.23). Using this value, the fluorescence quantum yield of the concentrated solution, Φ_F^{conc}, could be obtained from the ratio of the areas of the two spectra shown in Fig. 2a. Because monomers and a dimers coexisted in the concentrated solution, Φ_F^{conc} could be expressed as follows:

$$\Phi_F^{conc} = \frac{S_{conc}/F_{conc}}{S_{dil}/F_{dil}} \Phi_F^{dil} = \frac{(S_{conc}^M + S_{conc}^D)/(F_{conc}^M + F_{conc}^D)}{S_{dil}^M/F_{dil}^M} \quad (4)$$

where S_{dil} and S_{conc} are the spectral areas of the fluorescence spectra of the dilute and concentrated solutions, respectively. The absorption fractions of the dilute and the concentrated solutions are F_{dil} and F_{conc}, respectively. The fluorescence spectrum of the concentrated solution could be divided into contributions from the monomer and dimer components using the values of S_{conc}^M and S_{conc}^D, respectively, for the spectral areas and F_{conc}^M and F_{conc}^D, respectively, for the absorption fractions. For the dilute solution, the contribution of the dimer could be ignored, and thus $S_{dil} = S_{dil}^M$ and $F_{dil} = F_{dil}^M$, where S_{dil}^M is the spectral area for the monomer. For the concentrated solution, the absorption fraction can be expressed as $F_{conc} = F_{conc}^M + F_{conc}^D$. Using eq. (4), we calculated Φ_F^{conc} to be 0.070. The clear decrease of Φ_F at high concentrations is an indication of so-called concentration quenching. In conventional fluorescence measurements, the cause of concentration quenching of fluorescence is difficult to discern because the effect of reabsorption must be taken into account. In this context, it should be emphasized that the reabsorption effect was sufficiently suppressed in the present measurements that the decrease of fluorescence intensity observed here was not due to reabsorption effects but rather to the decrease of Φ_F itself. The fluorescence spectrum of the dilute solution was similar to that of the concentrated solution (Fig. 2a). This similarity indicates that the fluorescence of the concentrated solution was due primarily to the EY monomer; the fluorescence due to the EY dimer was very weak. This conclusion is consistent with the fact that dye dimers do not fluoresce (vide supra).

For quantitative analysis, we examined the Φ_F of the monomer fluorescence contained in the fluorescence of the concentrated solution. The fluorescence spectrum of the concentrated solution consists of the contributions from the monomer and dimer (vide supra). To consider the contribution of the process in which the monomers absorb light and then fluoresce in the absence of additional mechanisms such as energy transfers and chemical reactions, the fluorescence spectra of the dilute solution and the concentrated solutions were normalized by F_{dil}^M and F_{conc}^M, respectively. The two spectra were very similar (Fig. 2b). This similarity indicates that the fluorescence of the concentrated solution was attributable primarily to monomer fluorescence after absorption of light by the monomer. The implication is that the Φ_F of this process and of the dilute solution were comparable. Moreover, this similarity suggests that the energy transfer from the excited EY monomer to the ground state EY molecule was unimportant in the fluorescence process of the monomer in the concentrated solution. It should be noted that the slight difference between the two spectra was more apparent at relatively long wavelengths. This pattern was probably due to dimer fluorescence, as discussed in detail below.

The absorption spectrum of the EY dimer was shifted to slightly longer wavelengths compared to the spectrum of the EY monomer (Fig. 1b). We therefore expected that the fluorescence spectrum of the EY dimer would also be shifted to longer wavelengths compared with that of the monomer and that only the monomer fluorescence would appear at relatively short wavelengths. The spectrum of the differences between the fluorescence spectra of the concentrated and dilute solutions normalized at 520 nm was then normalized by F_{conc}^D to facilitate evaluation of the fluorescence quantum yield (Fig. 2c). This normalized difference spectrum seemed to be shifted by about 11 nm to longer wavelengths compared with the fluorescence spectrum of the monomer. In addition, there was a mirror image relationship with the absorption spectrum of the dimer (Fig. 1b). This fluorescence spectrum could therefore be assigned to the fluorescence from the EY dimer, and the spectral area (S_{conc}^D) of the fluorescence intensity due to the EY dimer could therefore be obtained from this spectrum. The fluorescence quantum yield of the EY dimer Φ_F^D could therefore be expressed as follows:

$$\Phi_F^D = \frac{S_{conc}^D/F_{conc}^D}{S_{dil}^M/F_{dil}^M} \Phi_F^{dil} \quad (5)$$

Using eq. (5), we estimated Φ_F^D to be 0.005.

Reliable measurement of the fluorescence spectrum of the xanthene dye dimer was a difficult task because of the difficulty of suppressing the reabsorption effect. Penzkofer and Leupacher have studied the fluorescence behavior of rhodamine 6G (Rh6G) as a function of dye concentration up to high concentrations by use of a specially designed optical configuration and an analytical method to carefully subtract reabsorption effects.[7] According to their results, the spectrum of the Rh6G dimer is shifted to longer wavelengths by about 14 nm relative to the fluorescence peak of the monomer. The analysis indicated that the Φ_F was 0.0006, and the estimated lifetime of the dimer excited state was 2.2 ps. Although fluorescence spectra were not directly obtained in that study, similar results were obtained in the present study.

Transient absorption

It has been reported that the EY monomer has a $T_n \leftarrow T_1$ absorption band due to a monomer triplet state (T_1) at around 1000 nm.[19] From quantitative measurements of this absorption, the relative efficiency of the population of T_1 can be estimated. Figure 3 shows transient absorption signals of 0.05 mM and 2 mM solutions observed at 900 nm. The signal intensity A_{TT} was normalized by the fraction of absorbed light (F_A^M) due to the EY monomer at 532 nm. It is clearly apparent that the normalized signals with and without dimers in solution (A_{TT} / F_A^M) were almost the same (Fig. 3). The fraction of light absorbed by the sample solution was the sum of monomer and dimer contributions (= $F_A^M + F_A^D$). Accordingly, the number of T_1 generated was proportional to the number of excited EY monomers. The implication is that the excited EY dimer relaxed without participating in T_1 generation. It is interesting to note that decay rate for 2 mM solution was significantly faster than that for 0.05 mM solution. This would be due to self-quenching of T_1 state by the ground state EY.

Mechanism of EY dimer relaxation

As discussed above, the τ_R^D of the EY dimer (10 ns) was estimated to be twice the τ_R^M of the EY monomer (5 ns). Because the Φ_F^D of the EY dimer was estimated to be 0.005, the fluorescence lifetime of the EY dimer (τ_F^D), was 50 ps because τ_F^D equals the product of τ_R^D and Φ_F^D. Moreover, the analysis of transient absorption due to the triplet state showed that T_1 was not populated through excitation of the EY dimer. This result indicates that the very short fluorescence lifetime (τ_F^D) of the EY dimer was not due to ISC but instead was due to very rapid non-radiative relaxation to the ground state.

We studied the excitation and relaxation processes of the EY dimer based on measurements of fluorescence spectra and fluorescence quantum yields. Several attempts have been made to use laser spectroscopy to study the relaxation processes of xanthene dye dimers. Previously, the lack of fluorescence from the dye dimer has been attributed to fast ISC into T_1, although there was little direct experimental evidence to support this hypothesis. A reduction of the energy gap between T_1 and the excited state generated by the dimerization was consistent with this speculation. Although this hypothesis is still mentioned to explain the absence of fluorescence from the dye dimer, such a fast ISC mechanism has been belied by transient absorption experimental results[13,14] as well as photothermal spectroscopic studies,[15] including the present results.

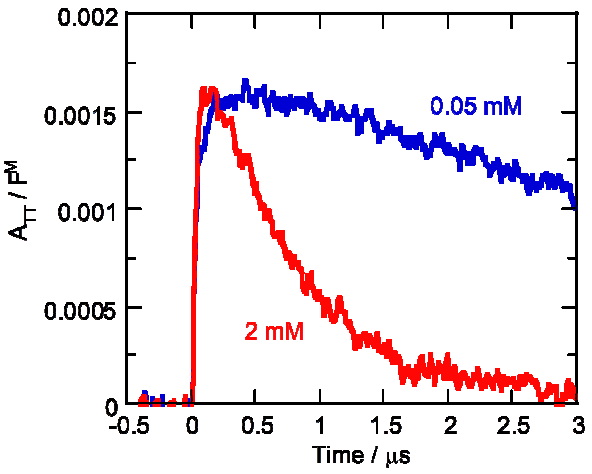

Fig. 3 Transient absorption signal recorded at 900 nm of EY at 0.05 mM and 2 mM in NaCl (1 M) aqueous solution normalized by the absorption fraction of the monomer.

Picosecond spectroscopic studies have indicated that the lifetime of the excited state of the RhB dimer is 100 ps,[13] similar to the fluorescence lifetime of 50 ps for the EY dimer estimated in the present study. Also, femtosecond transient absorption measurements performed on rhodamine 800 dimers have revealed a lifetime of about 3 ps.[14] A consistent explanation for the differences in the lifetimes of different dyes will be a focus of future research and will require a systematic examination of various dyes. Photo-thermal spectroscopy has also been applied to studies of the relaxation processes of dye dimers.[15] This method is a unique technique for detecting the temporal pattern of heat released during relaxation from excited states. For RhB and EY dimers, it has been pointed out that ISC and dissociation of excited dimers into two monomers is not an important relaxation process. The implication is that IC is an important mechanism for the relaxation of dye dimers. Based on these previous experimental results, including our results, we conclude that the failure of dye dimers to fluoresce is caused by fast IC.

When a fluorescent dye such as EY forms a dimer, relaxation processes from its excited state change dramatically. Understanding of such an ultra-fast IC mechanism is a challenging task for basic photo-physics. Relaxation processes involving molecular aggregates have been studied not only for dye dimers formed in aqueous solutions but also for densely adsorbed molecular systems. For example, in dye-sensitized solar cell systems, dyes are densely adsorbed on oxide semiconductor films. Intermolecular interactions therefore play an important role in relaxation processes from excited state. Recently, charge separation processes ($M + M \rightarrow M^+ + M^-$) between dyes on the surface after photoexcitation have been discussed.[20] After charge separation, non-radiative relaxation occurs rapidly through charge recombination processes. Such separation and recombination reactions between the same molecules have also been found in perylene excimers.[21] Such charge separation is thus considered to be an important relaxation process of molecular aggregates. For xanthene dyes, an electron transfer reaction from triplet excited dye to the ground state dye in solution has been discussed as a D-D mechanism.[22] Accordingly, charge separation between dyes of the dimer and subsequent rapid recombination is a possible model to explain fast IC in excited dimers. Unfortunately, no direct observations of such charge separated states in dye dimers has yet been reported. Use of ultrafast laser

spectroscopy would facilitate addressing this issue in the future.

Conclusions

In this study, we obtained fluorescence spectra of EY dimers using a newly developed experimental technique to eliminate reabsorption effects of fluorescence, even in samples containing a high concentration of dimers. The results obtained in this study are summarized schematically in Fig. 4.

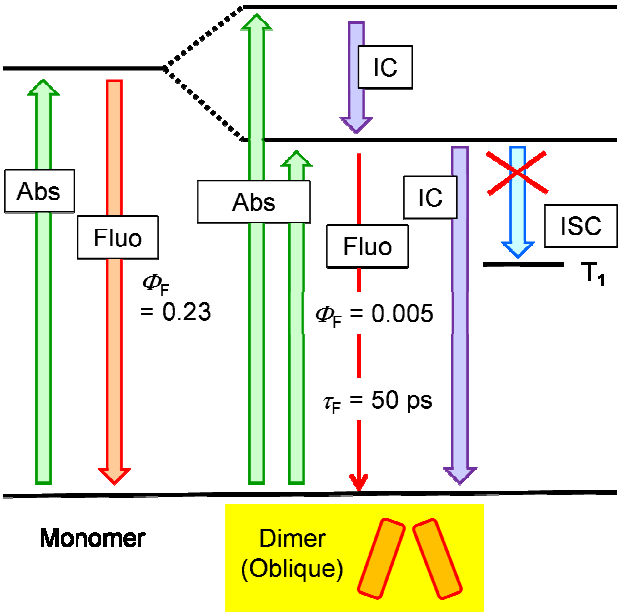

Fig. 4 Schematic diagram of the results obtained in this study.

From the analysis of the absorption spectra, we found that the EY dimer show two absorption peaks indicating oblique structure. Using a conventional technique, fluorescence quantum yields of the EY monomer was estimated to be 0.23. Using the value, fluorescence quantum yields and lifetimes of excited EY dimers were estimated to be 0.005 and 50 ps, respectively. Transient absorption was performed to study the relaxation channel from the excited EY dimer. We found that no evidence of triplet formation from the EY dimer in the excited state through intersystem crossing (ISC). This indicates that internal conversion (IC) is a dominant relaxation channel of the excited EY dimer.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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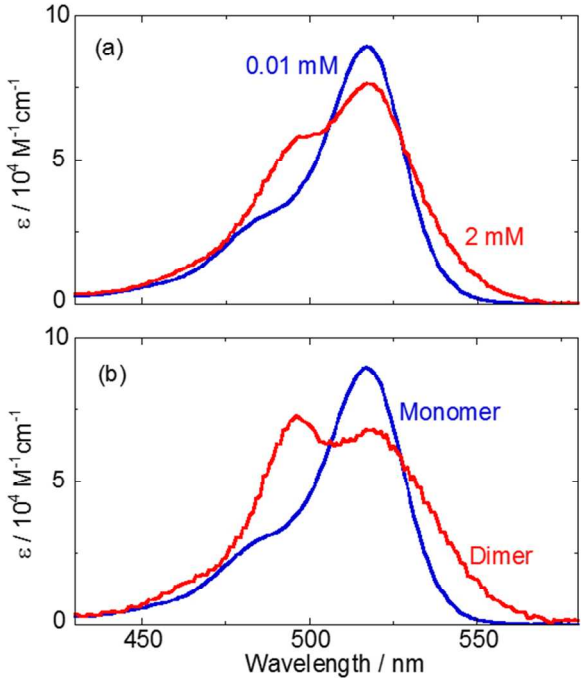

Fig. 1 Absorption spectra of EY at 0.01 mM and 2 mM in NaCl (1 M) aqueous solution (a). Monomer and dimer component of the absorption spectra (b).

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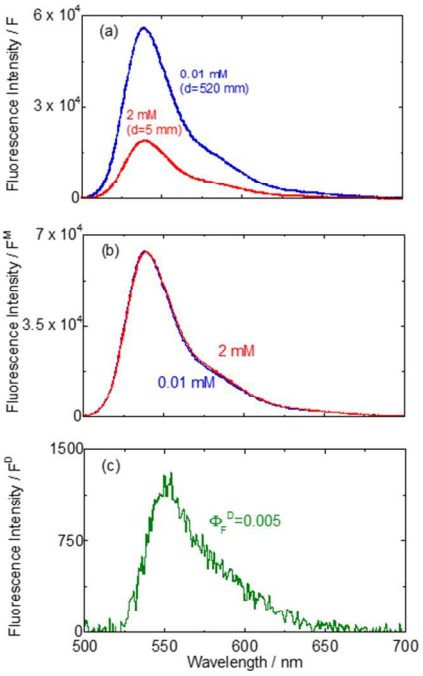

Fig. 2 Fluorescence spectra of EY at 0.01 mM and 2 mM in NaCl (1 M) aqueous solution normalized by the absorption fraction of the sample solution (a) and by the absorption fraction of the monomer (b). Difference spectrum between the fluorescence spectrum of a 2 mM solution and that of a 0.01 mM solution normalized at 520 nm (c).

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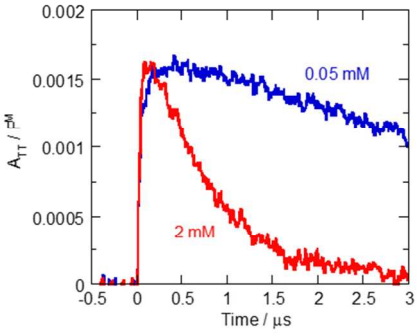

Fig. 3 Transient absorption signal recorded at 900 nm of EY at 0.01 mM and 2 mM in NaCl (1 M) aqueous solution normalized by the absorption fraction of the monomer.

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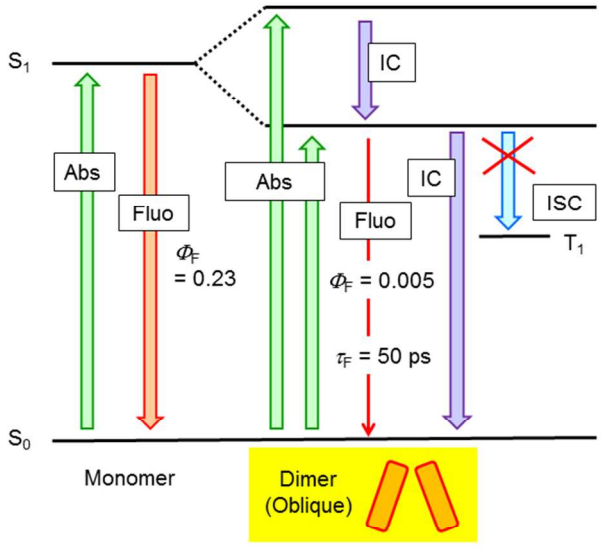

Fig. 4 Schematic diagram of the results obtained in this study.

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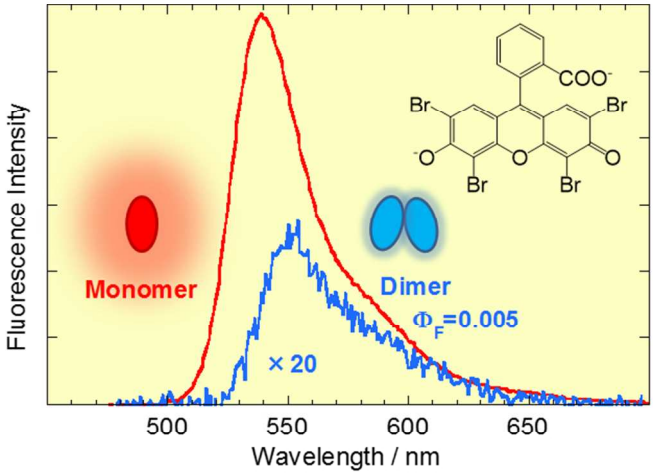
Weak fluorescence from Eosin Y dimer can be observed.
Fluorescence quantum yield was estimated to be 0.005.



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