PHASE-SENSITIVE FLUORESCENCE STUDY OF MONO-L-ASPARTYL CHLORIN E6

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ABSTRACT-Phase-resolved fluorescence studies of a photosensitizer for photodynamic therapy, mono-L-aspartyl chlorin e6 (ME2906), have been carried out. The experiments were done for its water solutions in the concentration range from 3.13x10^{-7} to 8.00x10^{-5} M, and some photophysical parameters have been experimentally determined for the lowest singlet excited state of ME2906. It was confirmed that ME2906 molecules were in the isolated molecular state below 1.00x10^{-5} M. It was also confirmed that the fluorescence in this concentration range was ascribed to the electronic transition from the lowest singlet excited state to the ground state. It was found that, above 1.00x10^{-5} M, a part of ME2906 molecules form dimers in water solution, which causes the red shift of fluorescence spectrum and the enhancement of fluorescence in the 700-750 nm wavelength region. Semiempirical molecular orbital calculation revealed that the sodium aspartate attached to the tetrapyrrole ring through the ethanoic acid group was remarkably bent with respect to the tetrapyrrole plane, which seems to hinder the formation of Me2906 dimers up to 1.00x10^{-5} M.

Keywords - mono-L-aspartyl chlorin e6,
phase-resolved fluorescence

INTRODUCTION

Photodynamic therapy (PDT) has been evolving as a modality for the treatment of malignant tumors. Most of the clinical work on PDT has been carried out, using hematoporphyrin derivative (HpD). [1,2] HpD is effective as a photosensitizer of PDT, but has some problems to be overcome. One of them is that HpD does not absorb photoradiation strongly in the red region of the spectrum where the radiation penetrates deeply into human tissues. Thus, extensive research has been done, looking for new photosensitizers that have optical absorption at longer wavelength, for example. A promising candidate is mono-L-aspartyl chlorin e6 (ME2906), where the double bond of the D ring in the porphyrin ring is reduced and long wavelength absorption is realized. [3] As a photosensitizer used in PDT, photophysical properties of its triplet state were reported by Spikes and Bommer. [4] Some preliminary studies were also reported on its singlet state such as fluorescence decay time. [5] However, photophysical information of ME2906 is very limited and systematic studies are only a few.

It is indispensable to acquire the detailed information on the dissipation processes of the photon energy absorbed by photosensitizers, which play essential roles in PDT, in order to have comprehensive understanding of PDT in a molecular scale. The purpose of this report is to present experimental results of phase-resolved fluorescence measurement on ME2906, which is currently an important photosensitizer in PDT. In this study, we have done the phase-resolved fluorescence measurement on ME2906 solutions in wide concentration range. We have discussed the nature of the emitting molecular state of ME2906, based on the results. In addition, the emitting molecular state has been discussed, being related with the molecular configuration of ME2906.

MATERIALS AND METHODS

Mono-L-aspartyl chlorin e6 (ME2906) is structurally a compound similar to porphyrin, but a sodium aspartate is attached via the peptide linkage to an ethanoic acid at the 15th position and a sodium propanoate at the 17th position of a tetrapyrrole ring. Its molecular weight is 799.69 [6]. Its chemical structure is given in Fig. 1 as the inset. In this study, ME2906 was dissolved in distilled water. Its concentration was varied from 3.13x10^{-7} to 8.00x10^{-5} M.

Excitation and fluorescence spectra of the ME2906 water solutions were measured with a SPEX Fluorolog-3 fluorescence spectrophotometer (New Jersey, USA) equipped with a Tau-3 fluorescence lifetime measurement unit. The fluorescence measurements were carried out in the back scattered and the normal 90º geometry to compare their results with one another and check the effect of self-absorption on the measurements. The effect was found not to be serious in this study. Fluorescence quantum efficiency was determined, using standard-grade perylene (Merck, Darmstadt, Germany) dissolved in ethanol as the standard of the efficiency. Its efficiency is 0.87 when excited at 367 nm. The absorption spectra of the ME2906 solutions and the ethanol solution of perylene were measured with the Shimadzu UV-2500 UV-VIS spectrophotometer (Kyoto, Japan) to determine their molar extinction coefficients.

The fluorescence decay time of the ME2906 water solutions was measured with the Fluorolog-Tau-3

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Abstract

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fluorescence measurement system in the frequency domain [7]. The rate constants of the radiative and the non-radiative deactivation-process were determined, using the results on quantum efficiency, η, and decay time, τ, of the fluorescence. The radiative (k_F) and the non-radiative (k_{NR}) rate constant were determined by the expressions, $k_F = \eta / \tau$, and $k_{ND} = (1-\eta) / \tau$, respectively.

RESULTS AND DISCUSSION

Excitation and Fluorescence Spectra

Typical results of the excitation and the fluorescence spectrum for ME2906 water solution are shown in Fig. 1, where ME2906 concentration is $2.50x10^{-6}$ M. The excitation spectrum was measured, detecting at 662 nm, and the fluorescence spectrum was measured, exciting at 400 nm.

The absorption peaks have been observed, for example, at 400, 502, 600 and 655 nm for ME2906 dissolved with phosphate buffer saline solution (PH = 7.4) [3]. The corresponding absorption peaks were observed in the excitation spectrum shown in Fig. 1. Basically, similar results were obtained in the concentration range from $3.13x10^{-7}$ to $1.00x10^{-5}$ M. The absorption peak at 400 nm tends to be smeared out above $1x10^{-5}$ M.

The emission peak is observed around 662 nm in the spectrum shown in Fig. 1. The emission wavelength was insensitive to ME2906 concentration up to $1.00x10^{-5}$ M. The normalized fluorescence spectra are shown in Fig. 2. They coincide with one another when ME2906 concentration is below $1.00x10^{-5}$ M, thus indicating that the emitting state involved is identical in this concentration range. The fluorescence intensity, defined as the area of the fluorescence spectra plotted against wavelength, is shown as a function of ME2906 concentration in Fig. 3. The solid circles stand for the experimental results. The solid line represents the linear dependence of the intensity upon ME2906 concentration. The intensity obtained in the present study is proportional to the concentration up to $1.00x10^{-5}$ M, and it tends to saturate above $1.00x10^{-5}$ M. These results clearly show that the emission observed blow $1.00x10^{-5}$ M is assignable to the electronic transition from the lowest singlet excited state of an isolated ME2906 molecule to its ground state.

The spectrum shown by solid circles in Fig. 2 is for the solution at $8.00x10^{-5}$ M. The emission wavelength was 673 nm at this concentration. The emission peak clearly shifts to the red as compared to those observed below $1.00x10^{-5}$ M. In addition, the mission in the 770-750 nm

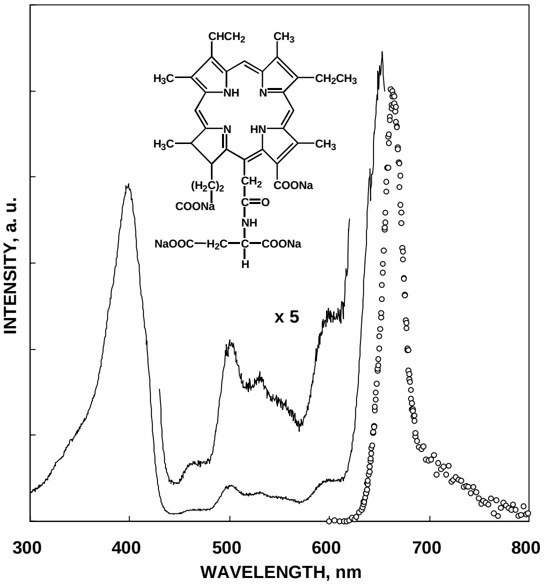

Fig:1 Excitation (solid lines) and fluorescence (open circles) spectrum of ME2906 water solution. The concentration of Me2906 is $2.50x10^{-6}$ M.

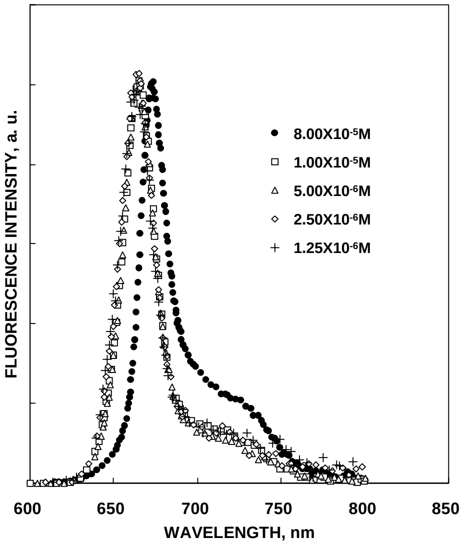

Fig:2 Normalized fluorescence spectra of ME2906 water solutions.

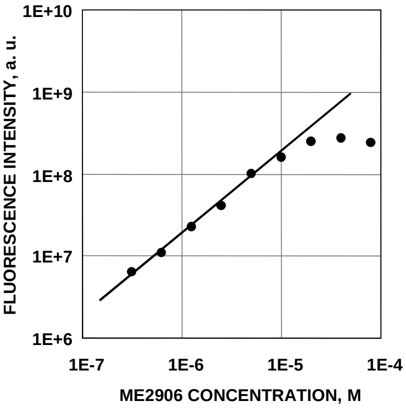

Fig:3 Fluorescence intensity as a function of ME2906 concentration. The solid circles stand for experimental results and the solid line for the linear relation between ME2906 concentration and fluorescence intensity.

wavelength region becomes more intense, as compared with those below 1.00x10^{-5} M. This strongly suggests that a part of ME2906 molecules form dimers above 1.00x10^{-5} M, as discussed later based on the results of the phase-resolved measurements.

Quantum Efficiency and Decay time of Fluorescence

The quantum efficiency and the decay time of the fluorescence are given as a function of the ME2906 concentration in Fig. 4. The solid squares and the circles represent the quantum efficiency and the decay time of the fluorescence, respectively. The quantum efficiency was 0.008-0.010 in the concentration range below 1.00x10^{-5} M, and is relatively insensitive to the concentration. It decreased rapidly above 1.00x10^{-5} M. It reduced to less than 2.0x10^{-3} at 8.00x10^{-5} M. Excitation wavelength dependence of fluorescence decay time was preliminarily investigated for some solutions to find that the decay time was almost independent of excitation wavelength. It indicates that the rate constant of the intersystem crossing process does not change so much and that the excited ME2906 molecules quickly relax to its lowest singlet excited state. The decay time was almost constant around 3.3 ns in the concentration range from 3.13x10^{-7} to 1.00x10^{-5} M. It showed a slight drop at 2.00x10^{-5} M and then gradually increased up to 3.8 ns at 8.00x10^{-5} M.

The rate constants of the radiative and the non-radiative processes, determined using the results of the quantum efficiency and the decay time of the fluorescence, are shown in Fig. 5 as a function of the ME2906 concentration. The non-radiative constant was 2.9-3.2x10^{8} s^{-1} and is almost constant in the concentration range investigated. On the other hand, the radiative constant was 2.5-3.1x10^{6} s^{-1} and was insensitive to the ME2906 concentration in the concentration range from 3.13x10^{-7} to 1.00x10^{-5} M, where the fluorescence intensity was proportional to the ME2906 concentration and the decay time is independent of the concentration. However, the rate constant decreased in the higher concentration range. It reduced to 4.0x10^{5} s^{-1}at 8.00x10^{-5} M.

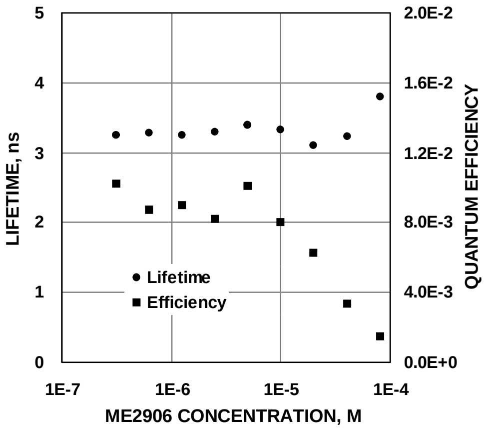

Fig:4 Quantum efficiency (solid squares) and decay time (solid circles) of fluorescence as a function of ME2906 concentration.

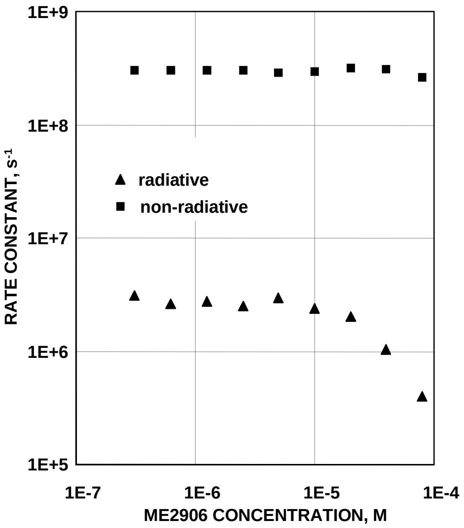

Fig:5 Rate constants of radiative (solid squares) and non-radiative (solid triangles) processes. These constants were calculated with equations shown in the text.

The fact that the rate constant of the radiative process is constant below 1.00x10^{-5} M coincides with the interpretation derived from the steady-state fluorescence measurement, which is that the fluorescence is ascribed to the electronic transition from the lowest singlet excited state of an isolated molecule to its ground state. The drastic change of the radiative rate constant above 1.00x10^{-5} M again coincides with the change of the normalized fluorescence spectra in the same concentration region observed in the steady-state measurement. Thus, the reduction of the radiative rate constant above 1.00x10^{-5} M indicates that an emitting state other than the monomeric singlet excited state contributes to the fluorescence of ME2906. A most probable possibility is that a part of ME2906 molecules form dimers, as indicated by the changes in the fluorescence spectra above 1.00x10^{-5} M.

Molecular Configuration

We carried out semiempirical molecular orbital (MO) calculation by the AM1 method to determine the optimized molecular configuration of ME2906 in the ground state and to have an insight into the experimental results obtained above. The calculation was done with WinMOPAC Ver.3 [8]. The COONa groups in ME2906 molecule were assumed in the calculation to be negatively ionized in water solution. For comparison, similar calculation was carried out for a hematoporphyrin molecule as well. The optimized conformation of ME2906 is given in Fig. 6(a). The gray, black and white balls stand for carbon, oxygen and nitrogen atoms, respectively. Hydrogen atoms are not shown in the figure for simplicity.

The tetrapyrrole ring of ME2906 is not so planar as that of hematoporphyrin. In addition, the sodium aspartate group attached to the tetrapyrrole ring through the ethanoic acid

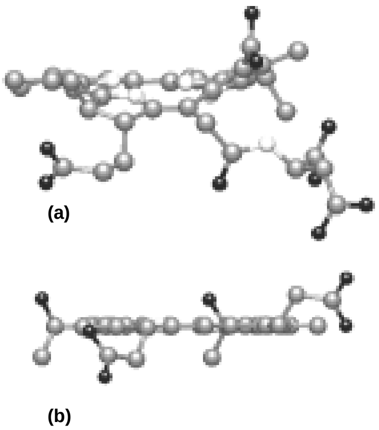

Fig:6 Optimized molecular configuration of ME2906 in the ground state. which is shown in (a). For comparison, similar calculation was done for hematoporphyrin, which is shown in (b).

group is remarkably bent with respect to the tetrapyrrole plane. This makes a striking contrast to a highly planar configuration of a hematoporphyrin molecule, which is shown in Fig. 6(b). The comparison of these optimized molecular configurations clearly reveals that ME2906 has much smaller possibility to form dimers, as compared with hematoporphyrin [9], because of the steric hindrance caused by the sodium aspartate group bent with respect to the tetrapyrrole plane. This is why the emission ascribable to an isolated molecule was observed for the ME2906 water solutions up to 1x 10^{-5} M. The possibility is nevertheless increased gradually for a pair of ME2906 molecules to form a dimer by coming closer to each other with the sodium aspartate group pointing outward and arranging their dipole moments in antiparallel, thus stabilizing the energy of the dimer. It seems that this takes place above 1.00x 10^{-5} M.

CONCLUSION

Photophysical properties of a photosensitizer, ME2906, for photodynamic therapy have been investigated with the phase-resolved fluorescence measurements. The parameters describing photophysical properties of its lowest singlet excited states have been experimentally determined for the first time. In addition, it was confirmed that ME2906 molecules were in the isolated molecular state below 1.00x10^{-5} M and that the fluorescence is ascribable to the electronic transition from the lowest singlet excited state of an isolated molecule to its ground state. It seems that, above 1.00x10^{-5} M, some molecules form dimers in water solution, which causes the red shift of the fluorescence spectrum and the enhancement of fluorescence in the 700-750 nm wavelength region. Semiempirical molecular orbital calculation revealed that the sodium aspartate group bonding to the tetrapyrrole ring through the ethanoic acid group was remarkably bent with respect to the tetrapyrrole plane, which seems to hinder the formation of a ME2906 dimer up to 1.00x10^{-5} M.

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