Efficiency Factors of Singlet Oxygen Generation from Core-Modified Expanded Porphyrin : Tetrathiarubyrin in Ethanol

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The photophysical properties and the singlet oxygen generation efficiency of tetrathiarubyrin have been investigated to elucidate the possibility of its use as a photodynamic therapy (PDT) photosensitizer by steady-state and time-resolved spectroscopic methods. The observed photophysical properties were affected by various molecular aspects, such as extended π conjugation, structural distortion, and internal heavy atom. The steady-state electronic absorption spectrum was red-shifted due to the extended π-conjugation, and the spin orbital coupling was enhanced by the structural distortion and the internal heavy atom effect. As a result of the enhanced spin orbital coupling, the triplet quantum yield increased to 0.90 ± 0.10 and the triplet state lifetime was shortened to 7.0 ± 1.2 μs. Since the triplet state decays at a relatively faster rate, the efficiency of the oxygen quenching of the triplet state decreases. The singlet oxygen quantum yield was estimated to be 0.52 ± 0.02, which is somewhat lower than expected. On the other hand, the efficiency of singlet oxygen generation during the oxygen quenching of triplet state, f_Δ^T, is near unity. Such high efficiency of singlet oxygen generation can be explained by the following two possible factors: The hydrogen bonding of ethanol which impedes the deactivation pathway of the charge transfer complex with oxygen to the ground state, the less probability of the aggregation formation.

Keywords: Tetrathiarubyrin, Triplet state, Singlet oxygen, Photophysics, Photodynamic therapy.

Introduction

Photodynamic therapy (PDT), a recently developed method for the treatment of tumors, has opened research on developing effective and selective photosensitizers. One of the most desirable properties is the red light absorption of photosensitizers since red light penetrates the tissue more deeply with minimal light loss to biological cells.[1,2] Core-modified expanded porphyrin is a new class of porphyrin-like photosensitizers that show such red-shifted absorption due to the extended π-conjugated system. The class of these compounds is actively being investigated for application to the photosensitizer due to such an advantage.[3] Other implicit factors are the nature of the excited state of the photosensitizer, the singlet oxygen generation efficiency, and biological factors, such as specific binding efficiency to cancer cells and nontoxicity.[4,5] The high singlet oxygen quantum yield is one of the most important photophysical requirements. The high triplet state quantum yield is also a prerequisite of the high singlet oxygen quantum yield. Besides the high triplet

quantum yield, other photophysics of the lowest triplet state, such as triplet state lifetime and triplet state quenching rate by oxygen, may affect profoundly the formation efficiency of the singlet oxygen.[6,7] This formation efficiency of the singlet oxygen can be expressed by several photophysical parameters concerning the triplet state and the singlet oxygen.

Tetrathiarubyrin, one of the core-modified expanded porphyrins, has a larger aromatic system (26π electrons) in which four pyrrole rings of its nitrogen analogue, rubyrin, are replaced by thiophene.[8] This modified molecular structure of tetrathiarubyrin is expected to lead to different photophysical properties compared with other photosensitizers due to the ring expansion and heavy-atom substitution. The ring expansion of this compound is considered to be the cause of the structural distortion as shown in the semi-empirical calculation[8] and, as a consequence, to promote triplet state quantum yield *via* the enhanced intersystem crossing rate, as with other nonplanar porphyrins. The incorporation of heavy atoms into the intramolecular structure of the photosensitizer, such as dialkylcarbocyanine dyes, increases the rate of intersystem crossing and the triplet state quantum yield.[9] From the expectation of high triplet state quantum yield, tetrathiarubyrin is supposed to have the high singlet oxygen quantum yield.

In the present work, we have investigated the photophysics of tetrathiarubyrin, especially of the triplet state, and measured the efficiency factors of singlet oxygen generation to provide information on determining the possibility of its use as a PDT photosensitizer, employing both steady-state and time-resolved spectroscopic methods.

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Experimental Section

Material. Ethanol (Merk, HPLC grade) was used as a solvent for all solutions without further purification. Partially degassed solutions were prepared by dissolving the sample in degassed ethanol, which was obtained by using the degasser (DEGASYS, DG-1210) under nitrogen environment (high purity, 99.999%). All air saturated solutions were prepared by bubbling with air gas (high purity, 99.99%) for 45 minutes. To preserve the gassing and degassing conditions, airtight quartz cuvettes were used for all experiments. The optical density of the recrystallized sample solution at the excitation wavelength in each experiment was adjusted to be less than 0.1. The structure of tetrathiarubyrin is depicted in Figure 1, and the detailed process of synthesis is reported in the reference.[10]

Photophysical measurements. Steady-state absorption measurement was carried out using a UV-VIS spectrophotometer (Jasco, V-530), and steady-state fluorescence spectra were measured with the spectrofluorimeter (Hitach, F-4500).

Time-resolved transient absorption was measured by a Q-switched neodymium yttrium aluminum garnet (Nd : YAG) laser-pumped optical parametric oscillator (OPO) laser (B. M. Industries, OP 901-355) delivering 5 ns pulses at 517 nm as an excitation source, and its excitation pulse energy was adjusted to be ~700 μJ/pulse. A tungsten lamp (home-made) was used as the probe beam, which was shaped by an iris and then focused perpendicularly onto the sample region, which was excited by the OPO laser. The transmitted beam was collected through a monochromator (Jobin Yvon, H20) and detected at a PMT (Hamamatsu, R928). The signal was fed to a 500 MHz digital oscilloscope (Hewlett Packard, 54520A) for digitizing and averaging. The data were fitted with a non-linear regression program on a personal computer.

The Q-switched Nd : YAG pumped OPO laser was also used as an excitation source for the time-resolved thermal lens method. The excitation pulse energy and wavelength were ~30 μJ/pulse and 517 nm, respectively. The collinear helium-neon (He-Ne) laser (Uniphase, 1103P; 2 mW) beam probed the thermal lens effect, which was induced in the sample solution. Two beams were combined by a Glan-Thomson polarizer (Melles Griot) and collimated. The intensity of the He-Ne laser beam at the center was monitored
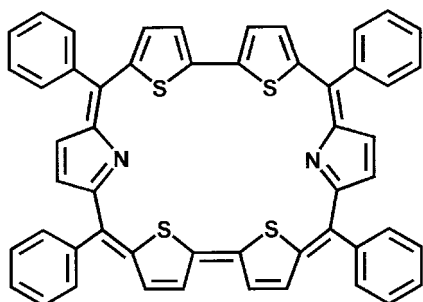
through a pinhole (300 μm diameter), which was placed at the center of the beam after the sample cuvette, and the monochromator, and PMT. The data were collected by a 500 MHz digital oscilloscope.

Results and Discussion

Steady-state absorption and fluorescence. As shown in Figure 2, the Soret band of tetrathiarubyrin is shifted to a wavelength longer than those of the common porphyrins, such as tetraphenylporphyrin (H$_2$TPP) and zinc tetraphenylporphyrin (ZnTPP),[11,12] and it is most intensely peaked at 517 nm. The largest of the Q bands is peaked at 714 nm. They are also shifted to a longer wavelength. Such red shift occurs due to the 26 π electrons, leading to a large conjugation effect (see Figure 1).

Steady-state fluorescence spectra of tetrathiarubyrin were measured at room temperature at the excitation of 517 nm. A very weak fluorescence band was observed at around 675 nm. The fluorescence quantum yield of this band was measured to be near zero ($\Phi_f \ll 0.001$). On the other hand, no emission was observed at the excitation of 714 nm.

Time-resolved transient absorption. Time-resolved transient absorption was measured at 650 nm under both partially degassed and air-saturated conditions. The signals were best fitted by double exponential decays. The microsecond transient absorption signals under both conditions are shown in Figure 3. The lifetimes of the two decay components under partially degassed condition were 370 ± 20 ns and 4.9 ± 0.1 μs, and the lifetimes became 220 ± 20 ns and 2.5 ± 0.1 μs under air-saturated condition. The lifetime of 370 ns component (oxygen quenched case: 220 ± 20 ns) under partially degassed condition may be due to a small amount of radical or protonated species that were produced chemically or photochemically.[8,13] The slow transient components of 4.9 μs and 2.5 μs, therefore, were assigned to be the triplet state relaxation under the respective solution conditions.

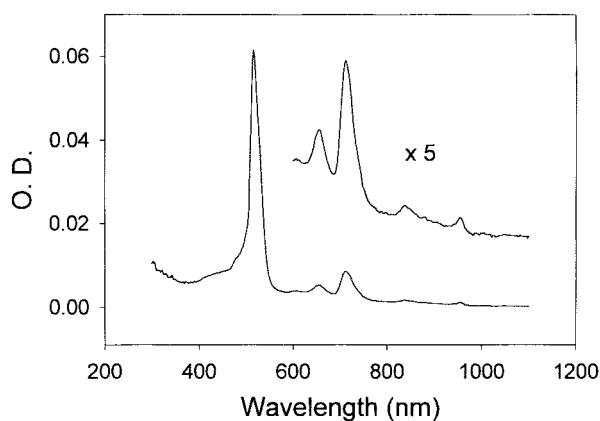**Time-resolved thermal lens**. Time-resolved thermal lens signal of singlet oxygen in air-saturated methanol solution,

Figure 1. Molecular structure of tetrathiarubyrin.

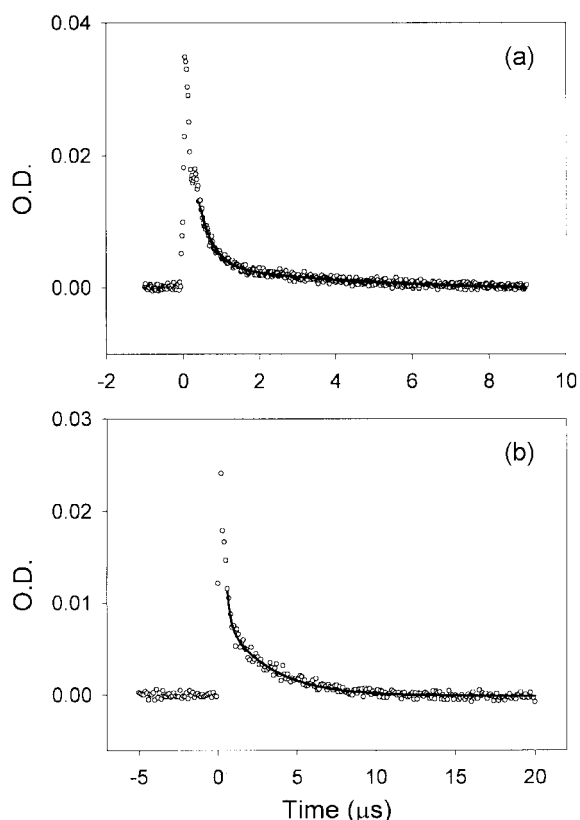**Figure 2**. Steady-state UV-VIS absorption spectrum of tetrathiarubyrin in ethanol.

Figure 3. Time-resolved transient absorption signals measured at 650 nm under (a) partially degassed and (b) air saturated conditions.
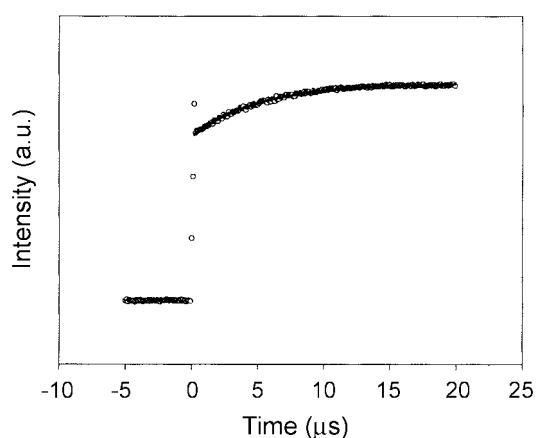

Figure 4. Time-resolved thermal lens signal of the singlet oxygen induced by hematoporphyrin in methanol under air saturated condition.

tracted from the total thermal lens signal to obtain the quantum yields and the lifetimes of the triplet state and singlet oxygen.[18] Therefore, in the present study, the time-resolved

which is obtained with hematoporphyrin, is utilized as a reference signal. Lifetime and quantum yield of the singlet oxygen generated by hematoporphyrin in the methanol solution are well known. As shown in Figure 4, the singlet oxygen lifetime and the quantum yield measured from our thermal lens setup are 6.0 ± 0.1 μs and 0.57 ± 0.01, respectively. The reported values are 7.0 μs and 0.53, which are nearly the same as our experimental values.[14,15] In general, the triplet state quantum yield (Φ_T) and the singlet oxygen quantum yield (Φ_Δ) are estimated from the following equations.[16]

$$\Phi_T = \frac{U_{S,T}}{U_T} \frac{h\nu_{ex} - \Phi_f E_f}{E_T} \tag{1}$$

$$\Phi_\Delta = \frac{U_{S,\Delta}}{U_T} \frac{h\nu_{ex} - \Phi_f E_f}{E_\Delta} \tag{2}$$

Here, U_T is the total amplitude of the thermal lens signal. $U_{S,T}$ and $U_{S,\Delta}$ are the slowly rising thermal lens amplitudes in partially degassed and air saturated conditions, respectively. ν_{ex} is the excitation frequency. Φ_f is the fluorescence quantum yield and E_f is the averaged fluorescence energy. E_T and E_Δ are the energies of the triplet state and the singlet oxygen, respectively.

Depending on the probe frequencies, the transient absorption signal at the He-Ne probe wavelength can be mixed with time-resolved thermal lens signal.[17] In this case, the component of the transient absorption signal must be sub-
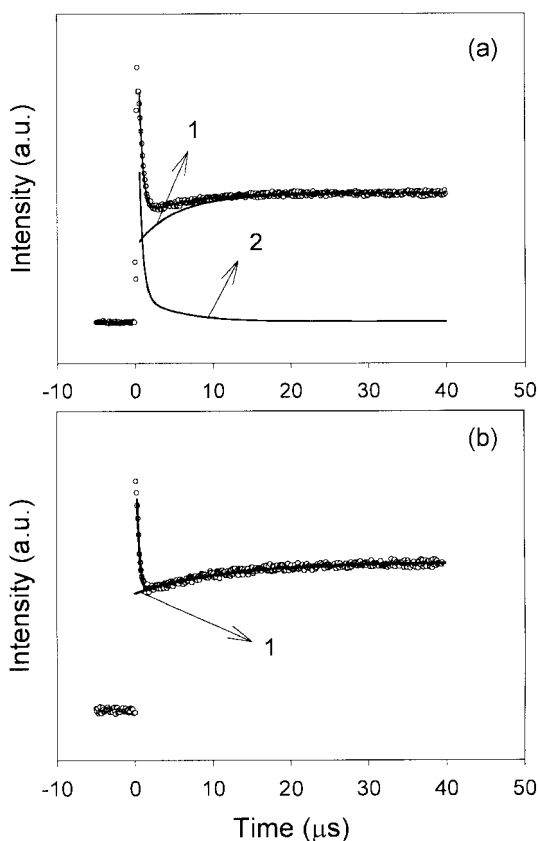

Figure 5. Typical time-resolved thermal lens data and the fitted lines under (a) partially degassed and (b) air saturated conditions: Line (a)-1 is the deconvoluted thermal lens signal of the triplet state relaxation in which the contribution of the transient absorption is subtracted, and line (a)-2 is the contribution signal of the transient absorption in the partially degassed condition; Line (b)-1 is the deconvoluted thermal lens signal of the singlet oxygen relaxation obtained by subtracting the contributions of the triplet state under air saturated condition.

transient absorption at the He-Ne probe wavelength was also measured and its contribution was subtracted from the total time-resolved thermal lens signal. In Figure 5, the total time-resolved thermal lens signals and the fitted lines under partially degassed and air saturated conditions are presented. From the subtracted thermal lens data, the lifetimes and the quantum yields of the triplet state and singlet oxygen were obtained.

The lifetime of the triplet state in the partially degassed condition is 4.9 ± 0.5 μs, which is consistent with the value obtained from the microsecond transient absorption measurement. Because of the instrumental limitation of the detection range, the phosphorescence could not be detected. The triplet state energy of this large aromatic compound is considered to be in near IR region and, therefore, the triplet state energy level may lie below 1000 nm as in other ring-expanded aromatic photosensitizers.[19] When this upper value of triplet state energy is substituted in Eq. (1), the triplet state quantum yield of tetrathiarubyrin is calculated to be greater than 0.8. This means that the triplet state quantum yield of tetrathiarubyrin is estimated to be between 0.8 and 1 (approximated value of 0.9). The triplet quantum yield depends generally on the intersystem crossing rate. Tetrathiarubyrin has an internal heavy atom, such as sulfur, and is known to be structurally distorted, as shown by semi-empirical calculations.[8] These factors are thought to promote the intersystem crossing rate through the enhanced spin-orbit coupling and, consequently, increase the triplet quantum yield.[9,20] The lifetime of singlet oxygen in air-saturated condition is measured to be 11.3 ± 0.6 μs, and the singlet oxygen quantum yield is estimated to be 0.52 ± 0.02, which is a rather low value compared with the high triplet quantum yield of 0.9.

Triplet state and singlet oxygen photophysics. The residual oxygen concentration, $[O_2]$, of the partially degassed condition is estimated to be 5×10^{-4} M from the measurement of the fluorescence intensity of anthracene in the same degassed condition. Since the fluorescence oxygen quenching rate and the lifetime of anthracene in ethanol were already known, the residual oxygen concentration could be estimated from the kinetic equation and the fluorescence intensity ratio. The oxygen concentration, $[O_2]'$, under the air-saturated condition is given to be 2.1×10^{-3} M.[21] The triplet lifetimes under partially degassed and air-saturated conditions were measured to be 4.9 ± 0.1 μs and 2.5 ± 0.1 μs, respectively.

$$k_T = k_T^0 + k_q[O_2] \tag{3}$$

$$k_T' = k_T^0 + k_q[O_2]' \tag{4}$$

The oxygen quenching rate constant of the triplet state (k_q) is estimated from Eq. (3) and (4), where k_T and k_T' are the measured decay rate constants of the triplet states under partially degassed and air-saturated conditions, respectively, and k_T^0 is the intrinsic decay rate constant of the triplet state.[22] By subtracting Eq. (3) from Eq. (4) and substituting two measured values of k_T's at two different oxygen concentrations, the oxygen quenching rate constant of the triplet state, k_q, is

calculated to be 1.2 (± 0.1) $\times 10^8$ M^{-1}s^{-1}, which is somewhat lower than the rate constants of other photosensitizers. The intrinsic triplet state lifetime is 7.0 ± 1.2 μs. The intrinsic triplet lifetime of tetrathiarubyrin is faster than those of other photosensitizing porphyrins. As mentioned previously, tetrathiarubyrin has relatively heavy internal core atoms and its conformational structure is suggested to be distorted, as shown by semiempirical calculations.[8] Therefore, this implies that these structural factors are considered to promote the nonradiative T_1 relaxation rate through the enhanced $T_1 \rightarrow S_0$, resulting in the faster triplet state lifetime.

The efficiency of singlet oxygen generation, f_Δ^T, induced by the energy transfer during the oxygen quenching of the triplet state is given by the following equations.[23,24]

$$\Phi_\Delta = \Phi_T P_{O_2}^T f_\Delta^T \tag{5}$$

$$P_{O_2}^T = \frac{k_q[O_2]'}{k_T^0 + k_q[O_2]'} \tag{6}$$

$P_{O_2}^T$ is the fraction of the triplet states quenched by oxygen,[23,24] and it is estimated to be 0.65 ± 0.06 under the air-saturated condition. This indicates that the triplet state is not completely quenched by oxygen even under the air-saturated condition due to the low triplet state quenching rate and the relatively faster triplet relaxation. Consequently, the singlet oxygen quantum yield is somewhat smaller compared with the high triplet state quantum yield due to such inefficient quenching of the triplet state by oxygen. On the other hand, f_Δ^T is calculated to be 0.89 ± 0.11 under the air saturated condition. Such a high value means that the triplet state quenched by the oxygen molecule generates mostly the singlet oxygen through the energy transfer process. f_Δ^T is known to be determined by various factors, such as, electron configuration, molecular structure, oxidation potential, etc.[25] Tetrathiarubyrin is expected to have a low oxidation potential as do other core-modified derivatives of rubyrin that show the oxidation potential value of 0.53-0.86 V.[26] Therefore, it has the high possibility of charge transfer exciplex formation with oxygen and the relaxation of its complex state without singlet oxygen generation.[27] However, for tetrathiarubyrin in ethanol, the efficiency of singlet oxygen generation (f_Δ^T) is high enough to be near unity. Such a high value of f_Δ^T of tetrathiarubyrin indicates that the aggregation formation, which usually decreases the energy transfer to oxygen,[28] is less probable in this molecular system. Besides the low probability of the aggregation formation, the solvation effect due to the hydrogen bonding between ethanol and tetrathiarubyrin can also be a factor in the high f_Δ^T value. Such solvation effect is reported to decrease the deactivation rate

Table 1. Photophysical parameters of triplet state and singlet oxygen

τ_T/μs (intrinsic)	Φ_T	$k_q/$ 10^8 M^{-1}s^{-1}	$P_{O_2}^T$	f_Δ^T	Φ_Δ
7.0 ± 1.2	0.90 ± 0.10	1.2 ± 0.1	0.65 ± 0.06	0.89 ± 0.11	0.52 ± 0.02

of the encounter complex with oxygen and, consequently, to increase the probability of the energy transfer to oxygen.[29]

Conclusions

In the present study, the photophysical properties of tetrathiarubyrin have been investigated with respect to the structural factors, which were the extended π conjugation, the structural distortion, and the internal heavy atom. The absorption spectrum was red-shifted due to the larger conjugated aromatic system. The Soret band was peaked at 517 nm and Q bands were located around 714 nm. The triplet state lifetime was shortened to 7.0 ± 1.2 μs and the triplet state quantum yield was enhanced to 0.9 ± 0.1. Such triplet photophysical properties are due to spin-orbit coupling enhancement caused by the structural distortion and the internal heavy atom.

The fraction of triplet states quenched by oxygen is calculated to be 0.65 ± 0.06 under the air-saturated condition, implying that the triplet state is not completely quenched by oxygen even under the air-saturated condition. The singlet oxygen quantum yield is 0.52 ± 0.02. This lower singlet oxygen quantum yield than the expected is due to the inefficient oxygen quenching of the triplet state which is caused by the low triplet state quenching rate and the relatively fast triplet state decay rate. However, the efficiency of singlet oxygen generation, f_{Δ}^T, of tetrathiarubyrin in ethanol is near unity despite the expected low oxidation potential. We suggest that the possible factors for such high efficiency of the singlet oxygen generation are the low probability of aggregation formation and the solvation effect of ethanol induced by hydrogen bonding which decreases the deactivation rate of the oxygen's encounter complex to ground state.

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