

Measurement of 1268 nm Emission for Comparison of Singlet Oxygen ($^1\Delta_g$) Production Efficiency of Various Dyes

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Singlet oxygen generation from laser-excited photosensitive dyes was measured directly using a sensitive near-infrared emission spectrometer to monitor the $O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-)$ transition at 1268 nm. The emission intensity was proportional to both the laser power and the concentration of the dyes. The singlet oxygen producing ability of the dyes was compared with that of eosin YS as a standard in methanol. The relative efficiencies of singlet oxygen generation were determined for rose bengal, erythrosine B, phloxine B and eosin YS as 2.39, 1.73, 1.38, 1.00, respectively, while uranine showed no emission in this spectral region. Using rose bengal, erythrosine B, phloxine B and eosin YS, the efficiency of singlet oxygen generation correlated with the photobleaching reaction rate of azo-dyes by these dyes, suggesting singlet oxygen to be a species responsible for causing the photobleaching of azo-dyes. The halogen substituent effect on the efficiency of singlet oxygen generation from laser-excited photosensitive dyes was also examined systematically.

Key words singlet oxygen; near-infrared emission; dye; photobleach

Singlet oxygen ($^1\Delta_g$), a highly reactive and toxic intermediate, plays an important role in many photochemical and photobiological processes¹⁾ and has been postulated to be a major species mediating photochemical action in biological systems.²⁾

Many attempts have been made to find evidence of singlet oxygen generation in various photosensitized reactions and enzymatic systems.³⁾ In most of them, the transient presence of singlet oxygen is deduced from monitoring weak visible chemiluminescence, chemical products, chemical scavenging and deuterium kinetic isotopic effects. Many of these secondary effects can equally indicate the presence of another reactive intermediate, O_2^- , $\cdot OH$, and also cannot distinguish between sigma and delta singlet oxygen.

Compared with these nonspecific techniques for the detection of singlet oxygen, direct observations of the $(0,0) \ ^1\Delta_g \rightarrow \ ^3\Sigma_g^-$ singlet oxygen emission at 1268 nm has proven to be the most reliable approach for singlet oxygen detection. Since the first observation of photosensitized singlet oxygen emission in solution,⁴⁾ the emission measurement has been used for the detection and investigation of singlet oxygen in photochemical and biological systems.⁵⁾

Rose bengal and its derivatives are often used for cosmetics and foods. However these dyes are known to be photosensitive and phototoxic, and to accelerate the photobleaching of other dyes. Additionally, photooxidation of ingredients by these dyes is often observed in the products. The nature of the active species causing photobleaching and photooxidation has not been established, although activated oxygen species have been postulated.

We have recently constructed a sensitive near-infrared emission spectrometer with a germanium (Ge) detector which can scan from 1000 to 1600 nm. We applied this apparatus for the detection of singlet oxygen produced by laser-excited photosensitive dyes. In this work, we first

measured the near-infrared emission spectra corresponding to the $O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-)$ transition of singlet oxygen in various photosensitive dye solutions on laser irradiation. The experiments were performed using a sensitive near-infrared spectrometer to quantitatively monitor emission intensity due to the $O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-)$ transition at 1268 nm.

We also report and discuss the singlet oxygen generation ability of rose bengal derivatives from the halogen substituent effect.

Experimental

Materials Five dyes were used in this investigation: eosin YS (tetrabromofluorescein sodium salt), erythrosine B (iodoeosine sodium salt), phloxine B, rose bengal, and uranine. Rose bengal was from Wako Pure Chemical Co. Eosin YS, erythrosine B, phloxine B, uranine and orange II (4-[(2-hydroxy-1-naphthalenyl)azo]-benzenesulfonic acid monosodium salt) were from Tokyo Kasei Kogyo Co., Ltd. (Japan).

Methanol (CH_3OH ; Wako Pure Chemical Industries, Ltd., Japan) was used as the dye solvent.

Singlet Oxygen Emission Measurements Our experimental setup (Fig. 1) consisted of argon (Ar) laser equipment (Innova 70-4; Coherent Inc., U.S.A.) and a near-infrared Ge detector (model 403HS; Applied Detector Co., U.S.A.) cooled by liquid nitrogen, connected to the exit slit of a monochromator (model CT10; JASCO, Japan) with a blaze wavelength at 1250 nm to minimize the grating loss. An IR-80 cutoff filter with 0% transmittance at less than 750 nm and 35% transmittance at 800 nm was placed at the entrance slit of the monochromator. A collecting lens focused the monochromator output onto the detector crystal. The Ar laser output at 514.5 nm was chopped with 800 Hz by an acousto-optic modulator (A-160; HOYA, Japan) driven by a driver (110-DS; HOYA, Japan). The signal output from the Ge detector was fed into a model 124A lock-in amplifier via a model 116 preamplifier (both from E.G. & G. Princeton Applied Research, U.S.A.) and synchronized with the internal reference signal of the lock-in amplifier. The signal output from the lock-in amplifier was fed to an XY recorder, and the emission spectrum was recorded by scanning the grating with a motor. In order to minimize photobleaching of the dyes, the solution was circulated by a peristaltic-pump through a quartz flow cell (3 × 3 mm).

Photobleaching of Orange II in the Presence of Rose Bengal Derivatives The photobleaching of orange II in the presence of rose bengal derivatives was estimated by monitoring the change in absorption of orange II at 484 nm (λ_{max}). The mixture of orange II (50 μM) and each rose bengal

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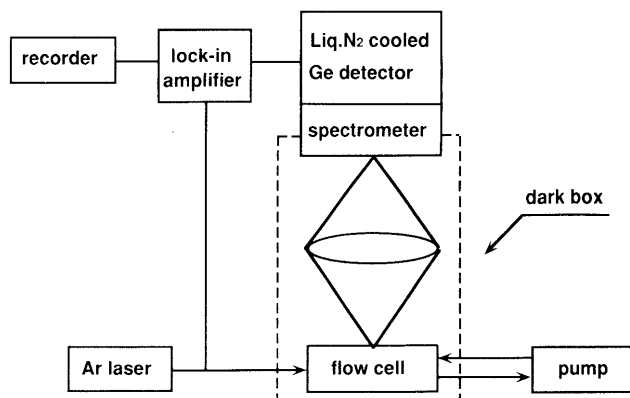


Fig. 1. Schematic Diagram of the Near-Infrared Spectrometer

derivative ($5\ \mu\text{M}$) methanol solution was set up in an FL-box at 20°C and was measured periodically with a Hitachi U-3210 spectrophotometer ($\lambda = 484\ \text{nm}$, cell length $1\ \text{cm}$).

Results

Near-Infrared Emission Spectrum Due to Singlet Oxygen Generated with Dyes as a Photosensitizer Figure 2 shows a near-infrared emission spectrum of rose bengal methanol solution excited with an Ar laser light at $514.5\ \text{nm}$, corresponding to the $\text{O}_2(^1\Delta_g) \rightarrow \text{O}_2(^3\Sigma_g^-)$ transition, obtained by using the apparatus shown in Fig. 1. The scanning time for the spectrum was about $15\ \text{min}$ with a slit of $2\ \text{mm}$ for both the entrance and exit slits of the monochromator with a grating blaze at $1250\ \text{nm}$. We can confidently assign the narrow band at $1268\ \text{nm}$ to the $(0,0)$ vibronic band of the $\text{O}_2(^1\Delta_g) \rightarrow \text{O}_2(^3\Sigma_g^-)$ transition. When dioxygen gas was bubbled in the solution, the spectrum had a peak at $1268\ \text{nm}$, but the spectrum disappeared when nitrogen gas was bubbled in. These results confirm that singlet oxygen is responsible for the emission at $1268\ \text{nm}$.

Near-infrared spectroscopic studies in the wavelength region of $1268\ \text{nm}$ were performed on the other dye solutions in the same way as described above. The emission of singlet oxygen was detected in erythrosine B, phloxine B and eosin YS methanol solution as well. Uranine methanol solution shows no emission in this spectral region. The singlet oxygen emission spectra in rose bengal and erythrosine B methanol solution do not overlap with any fluorescence emission of these dyes, while the singlet oxygen emission spectra of phloxine B and eosin YS methanol solution overlapped with their fluorescence emissions.

Effect of Laser Power and Rose Bengal Concentration on $1268\ \text{nm}$ Emission Intensity Figure 3 shows the relationship between the emission intensity at $1268\ \text{nm}$ in rose bengal solution and laser power. The emission intensity of singlet oxygen in laser-excited rose bengal increased with an increase in laser power at the range between 50 and $250\ \text{mW}$.

Figure 4 shows the dependence of the emission intensity at $1268\ \text{nm}$ on the concentration of rose bengal. The absorbance spectra of rose bengal in the same concentration (from 5×10^{-6} to $25 \times 10^{-6}\ \text{M}$) changed dose-dependently, and the intensity of the absorbance at $514.5\ \text{nm}$ was proportional to the concentration of rose bengal (Fig. 5). This trend was observed in methanol

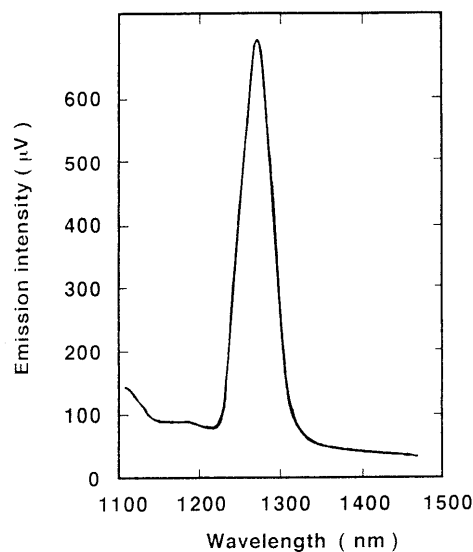


Fig. 2. Near-Infrared Singlet Oxygen Luminescence Emission Spectrum in Rose Bengal Methanol Solution ($10\ \mu\text{M}$) Excited by Ar Laser Light at $514.5\ \text{nm}$ with $200\ \text{mW}$ Output Power

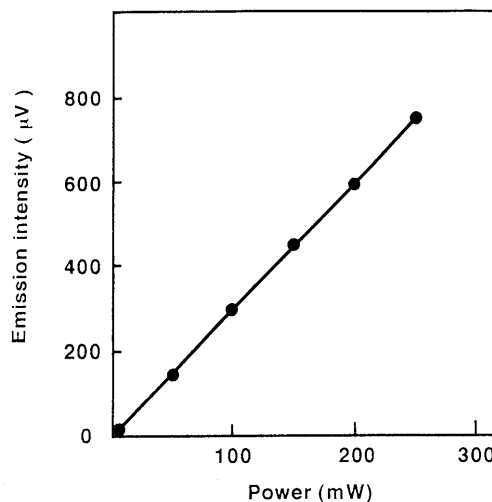


Fig. 3. Effect of Laser Power on the Singlet Oxygen Emission at $1268\ \text{nm}$ from Rose Bengal Solution

Singlet oxygen generation in rose bengal methanol solution ($10\ \mu\text{M}$) excited by Ar laser light at $514.5\ \text{nm}$ was monitored by measuring the emission intensity at $1268\ \text{nm}$.

solutions of erythrosine B, phloxine B and eosin YS as well. These results indicate that rose bengal derivative exists as a monomer, the interaction between molecules is almost negligible in the methanol solution of rose bengal derivatives (from 5×10^{-6} to $25 \times 10^{-6}\ \text{M}$), and singlet oxygen generation is largely a result of rose bengal derivative excitation.

Comparison of Emission Intensity Due to Rose Bengal Derivatives Table 1 shows the comparison of emission intensity among these solutions at $1268\ \text{nm}$. The values were calibrated by a molar extinction coefficient and then normalized by the value of the eosin YS solution. The efficiencies (I/ϵ) of singlet oxygen generation were determined for rose bengal, erythrosine B, phloxine B and eosin YS as 0.0189 , 0.0137 , 0.0109 , 0.0079 , respectively. The efficiencies (I/ϵ) of singlet oxygen generation were in the order of rose bengal > erythrosine B > phloxine B > eosin YS >> uranine. The relative singlet oxygen generation, estimated from this method, clearly indicates

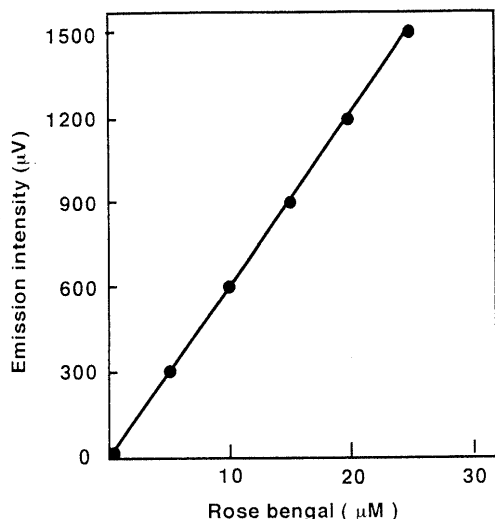


Fig. 4. Effect of Rose Bengal Concentration on the Singlet Oxygen Emission at 1268 nm from Rose Bengal Solution

Singlet oxygen generation in rose bengal methanol solution (5–25 μM) excited by Ar laser light at 514.5 nm with 200 mW output power was monitored by measuring the emission intensity at 1268 nm.

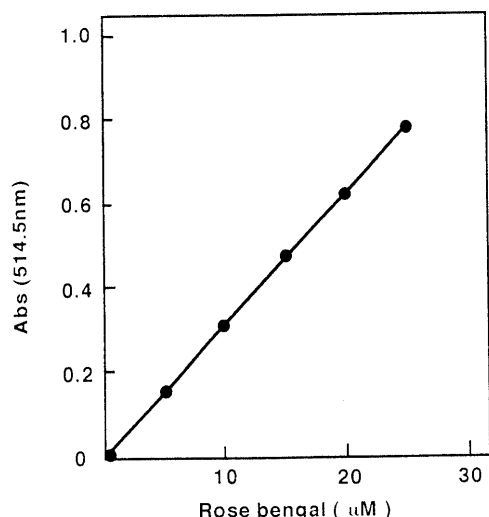


Fig. 5. Relationship between the Absorbance at 514.5 nm and the Concentration of Rose Bengal

Table 1. Comparison of Relative Emission Intensities at 1268 nm in Solutions of Various Dyes

Dyes	$A_{514.5}$	$\epsilon_{514.5}$	$^1\text{O}_2$ emission (I)	I/ϵ	I/ϵ (rel.)
Rose bengal	0.3148	31480	595	0.0189	2.39
Erythrosine B	0.4370	43700	600	0.0137	1.73
Phloxine B	0.3112	31120	340	0.0109	1.38
Eosin YS	0.6451	64510	510	0.0079	1.00

The emission intensity at 1268 nm was measured in the methanol solution of a dye at the concentration of 10 μM, excited by Ar laser light at 514.5 nm with 200 mW output power.

that the halogen substituted dyes are potent photosensitizers. These differences in singlet oxygen generation may be ascribed to the differences in conversion efficiency from the excited singlet state of the dye to the triplet state and within the lifetime of the triplet state. The triplet state of the halogen substituted dyes may be efficiently quenched by the ground state oxygen ($^3\Sigma_g^-$). The halogen

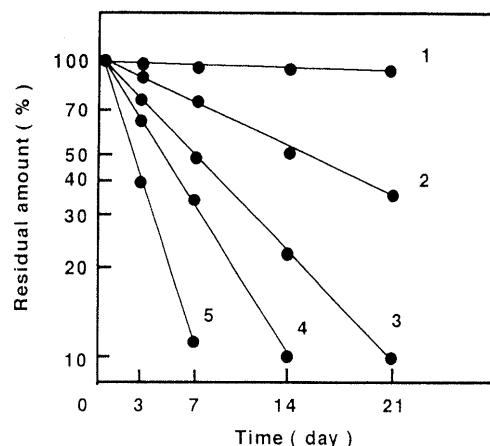


Fig. 6. Effect of Eosin YS, Phloxine B, Erythrosine B and Rose Bengal on the Photobleaching of Orange II

Line 1, no additives; line 2, 5 μM eosin YS; line 3, 5 μM phloxine B; line 4, 5 μM erythrosine B; line 5, 5 μM rose bengal.

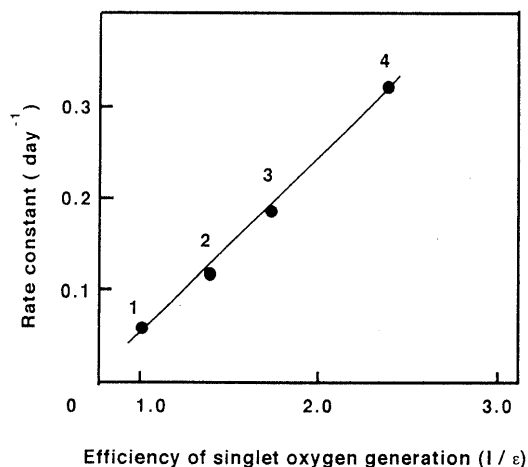


Fig. 7. Relationship between the Efficiencies (I/ϵ) of Singlet Oxygen Generation of Eosin YS, Phloxine B, Erythrosine B and Rose Bengal and Rate Constant of Photobleaching of Orange II

The values were calibrated by a molar extinction coefficient and then normalized by the value of the eosin YS solution. Circle 1, eosin YS; circle 2, phloxine B; circle 3, erythrosine B; circle 4, rose bengal.

substituent dyes were shown to generate singlet oxygen efficiently upon photoirradiation.

Photobleaching of Orange II in the Presence of Rose Bengal Derivatives Figure 6 shows the photobleaching of orange II in the presence of rose bengal derivatives in methanol solution for 3 weeks. Orange II did not photobleach in the absence of rose bengal derivatives, but this dye rapidly photobleached in the presence of rose bengal derivatives and dioxygen gas. The relationship between the efficiencies (I/ϵ) of singlet oxygen generation of these dyes and photobleaching rate is shown in Fig. 7. The efficiency (I/ϵ) of singlet oxygen generation is found to parallel the photobleaching rate of orange II by these rose bengal derivatives. These effects of rose bengal derivatives on photobleaching were observed in other azo-dyes, suggesting that singlet oxygen generation incites their photobleaching effects on azo-dyes.

Discussion

In this work, we first measured the direct near-infrared emission spectrum corresponding to the $\text{O}_2(^1\Delta_g) \rightarrow \text{O}_2(^3\Sigma_g^-)$ transition of singlet oxygen in solutions of

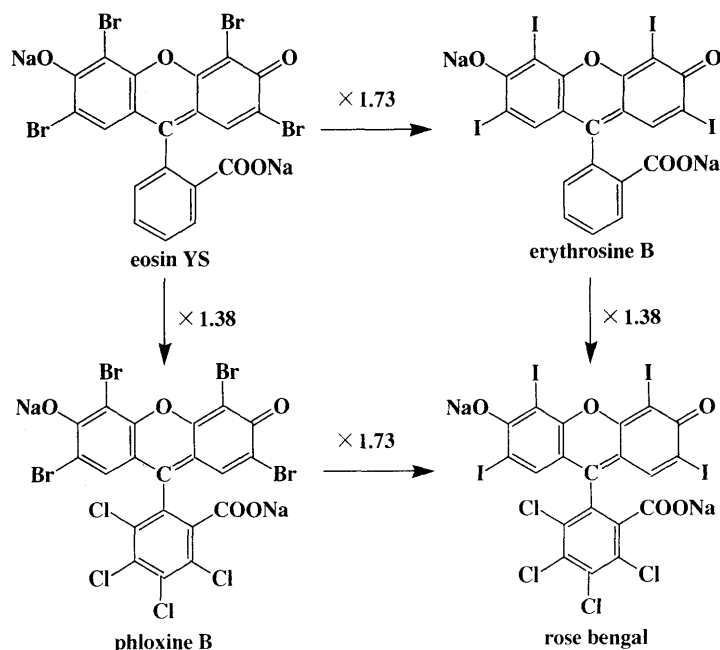


Chart 1

several rose bengal derivatives upon laser irradiation. The emission dependence on the intensity of laser power and the concentration of the rose bengal derivatives suggests that the derivatives generate singlet oxygen quite efficiently on photoirradiation over a wide concentration range. The photogeneration of singlet oxygen involves the transfer of excitation energy from an excited sensitizer molecule to ground state dioxygen ($^3\Sigma_g^-$). The dominant route of this electronic energy transfer is *via* the triplet state of the photoexcited sensitizer molecule.⁶⁾ The efficiency of singlet oxygen generation is very much dependent on the properties of the triplet excited state. In a halogen substituent series of aromatic molecules, the photopopulation of the triplet state follows the order $H < Cl < Br < I$, resulting from the mixing of electronic singlet and triplet states.⁷⁾ This expectation is consistent with the observed higher efficiency of singlet oxygen generation for rose bengal, erythrosine B, phloxine B and eosin YS compared to uranine.

We also compared the efficiency (I/ϵ) of singlet oxygen generation of compounds with halogen substitution (rose bengal, erythrosine B, phloxine B and eosin YS). The efficiencies of singlet oxygen generation of the Cl substituent series (phloxine B and rose bengal) are 1.38-fold higher than those by the non-substituent series (eosin YS and erythrosine B), and the efficiencies of singlet oxygen generation of the I substituent series (erythrosine B and rose bengal) are 1.73-fold higher than those by the Br substituent series (eosin YS and phloxine B) (Chart 1). We clearly indicated a halogen substituent effect on the efficiency of singlet oxygen generation from laser-excited photosensitive dyes.

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