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Comparison of Singlet Oxygen Production Efficiency of C_{60} with Other Photosensitizers, Based on 1268 nm Emission

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Singlet oxygen generation from laser-excited C_{60} was measured directly by 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 C_{60}. When nitrogen gas was bubbled into the C_{60} solution, the spectrum disappeared. Dose-dependent quenching of singlet oxygen by β-carotene was observed by monitoring the 1268 nm emission. Based on the quenching, the rate constant of the reaction of singlet oxygen with β-carotene was determined to be $1.5—2.3\times10^{10}\,\text{M}^{-1}\,\text{s}^{-1}$, which is consistent with the reported value ($1.5—2.5\times10^{10}\,\text{M}^{-1}\,\text{s}^{-1}$). A comparison of the singlet oxygen-producing ability of C_{60} as a photosensitizer with those of rose bengal, eosin and methylene blue, revealed C_{60} to have considerably higher efficiency.

Keywords singlet oxygen; C_{60}; fullerene; photosensitizer; near-infrared emission

Singlet oxygen ($^1\Delta_g$), a highly reactive and toxic intermediate, plays an important role in many photochemical and photobiological processes[1] and has been postulated to be a major species mediating photochemical action in biological systems.[2] Erythropoietic protoporphyria, tetracycline phototoxicity and photodynamic therapy of cancer are known to involve singlet oxygen as a species responsible for the action processes.[3] Many attempts have been made to find evidence of singlet oxygen generation in various photosensitized reactions and enzymatic systems.[4] Most of the investigations were based upon monitoring weak visible chemiluminescence, chemical products, chemical scavenger techniques and deuterium kinetic effects. Compared with these nonspecific techniques for the detection of singlet oxygen, direct observation 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,[5] emission measurement has been widely used for detection and investigation of singlet oxygen in photochemical and biological systems.[6]

The recent frenzy surrounding the fullerenes has encouraged investigators to elucidate fully the properties of C_{60}. In particular, the electronic properties of this unique π-system have attracted the attention of both photochemists and theoreticians. Based on photoacoustic,[7] thermal lensing[8] and singlet oxygen luminescence studies,[9] C_{60} has been postulated to produce singlet oxygen by energy transfer with unit efficiency. Foote and co-workers have conducted detailed studies on singlet oxygen generation in C_{60} solution using a kinetic apparatus based on a Ge-detector monitoring 1270 nm emission through interference filters.[9,10]

We have recently constructed a sensitive near-infrared emission spectrometer with a Ge-detector, which can scan from 1000 to 1600 nm. We applied this apparatus for detection of singlet oxygen produced by laser-excited C_{60}. Since the most difficult problem in time-resolved measurement of 1270 nm emission using interference filters is to distinguish the singlet oxygen phosphorescence from the near-infrared tail of the dye fluorescence and from the near-infrared emission due to temperature change, a better approach for comparison of singlet oxygen production efficiency of photosensitizers should be to acquire the signal due to the $O_2(^1\Delta_g)\rightarrow O_2(^3\Sigma_g^-)$ transition at 1268 nm by scanning the near-infrared wavelength region.

In this work, we firstly measured the near-infrared emission spectrum corresponding to the $O_2(^1\Delta_g)\rightarrow O_2(^3\Sigma_g^-)$ transition of singlet oxygen in C_{60} solution under laser excitation. The experiments were performed using a sensitive near-infrared spectrometer to monitor quantitatively the emission intensity due to the $O_2(^1\Delta_g)\rightarrow O_2(^3\Sigma_g^-)$ transition at 1268 nm. The essential parameter of a photosensitizer is its photodynamic efficiency, which is usually associated with a high quantum yield of singlet oxygen generation. In this paper, we also report the singlet oxygen-generating ability of C_{60} compared with that of other sensitizers.

Experimental

Materials Benzene (Wako Pure Chemical Industries Ltd., Japan), and methanol (Wako Pure Chemical Industries Ltd., Japan) were used as solvents for photosensitizers. C_{60} was purified by using HPLC with an ODS column (eluting solvents: toluene/methanol = 55/45). Rose bengal (RB) was from Wako Pure Chemical Co. Methylene blue (MB) and eosin yellowish (tetrabromofluorescein sodium salt) (EO) were from Tokyo Kasei Kogyo Co., Ltd., Japan.

Singlet Oxygen Emisson Measurement Our experimental set up (Fig. 1) consisted of an argon (Ar) laser (Innova 70-4; Coherent Inc., U.S.A.) and a near-infrared Ge-detector (mode 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 cut off 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 at 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 to a model 124A lock-in amplifier *via* a model 116 preamplifier (both from E. G. & G. Princeton

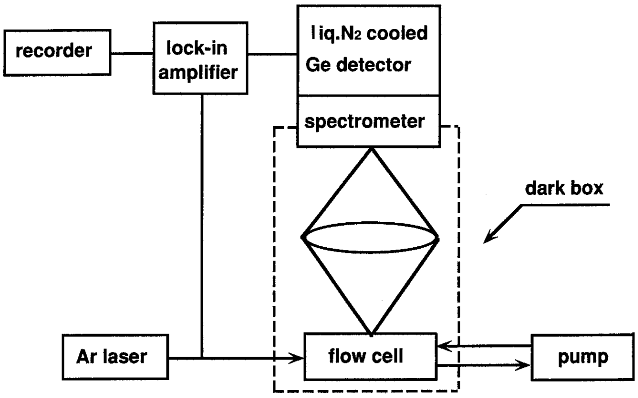

Fig. 1. Schematic Diagram of Our Near-Infrared Spectrometer

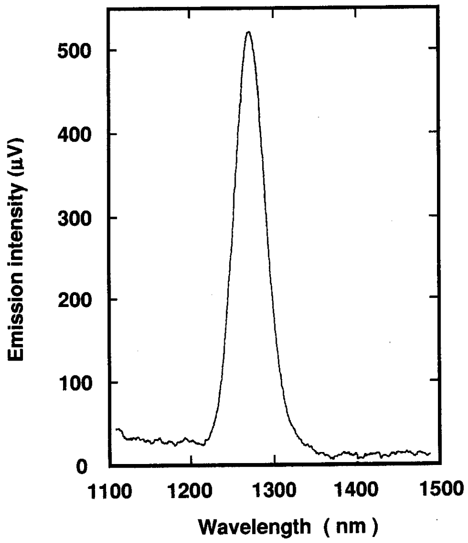

Fig. 2. Near-Infrared Singlet Oxygen Luminescence Emission Spectrum in C_{60} Benzene Solution (20 μM) Excited by Ar Laser Light at 514.5 nm with 200 mW Output Power

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 dye, the solution was circulated by a peristaltic pump through a quartz flow cell (3 × 3 mm).

Results

Near-Infrared Emission Spectrum Due to Singlet Oxygen Generated with C_{60} as a Photosensitizer Figure 2 shows a near-infrared emission spectrum of C_{60} benzene solution excited with Ar laser light at 514.5 nm, corresponding to the $O_2\ (^1\Delta_g)\rightarrow O_2(^3\Sigma_g{}^-)$ transition, obtained by using the apparatus shown in Fig. 1. The scanning time for the spectrum was about 15 min with a 2 mm slit for both the entrance and exit slits of the monochromator with a grating blaze at 1250 nm. We can confidently assign the narrow band at 1268 nm to the (0,0) vibronic band of the $O_2(^1\Delta_g)\rightarrow O_2(^3\Sigma_g{}^-)$ transition. When dioxygen gas bubbles were present in the solution, the spectrum had a peak at 1268 nm, but the spectrum disappeared when nitrogen gas was bubbled in. These results confirm that singlet oxygen is responsible for the emission at 1268 nm.

The C_{60}-sensitized singlet oxygen emission spectrum in benzene solution did not show any overlap with fluorescence of C_{60}, in contrast to the cases of hematoporphyrin, MB and EO.

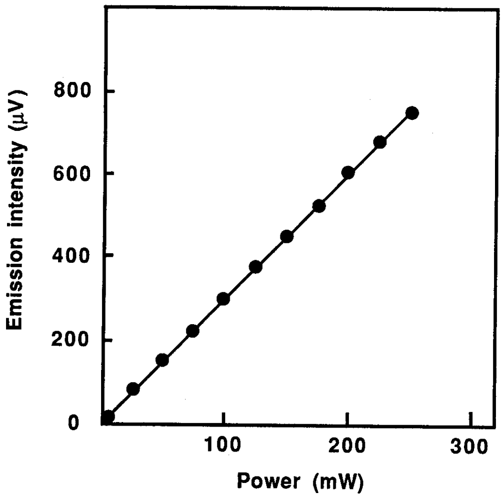

Fig. 3. Effect of Laser Power on the Singlet Oxygen Emission at 1268 nm from C_{60} Solution

Singlet oxygen generation in C_{60} benzene solution (25 μM) excited by Ar laser light at 514.5 nm was monitored by measuring the emission intensity at 1268 nm.

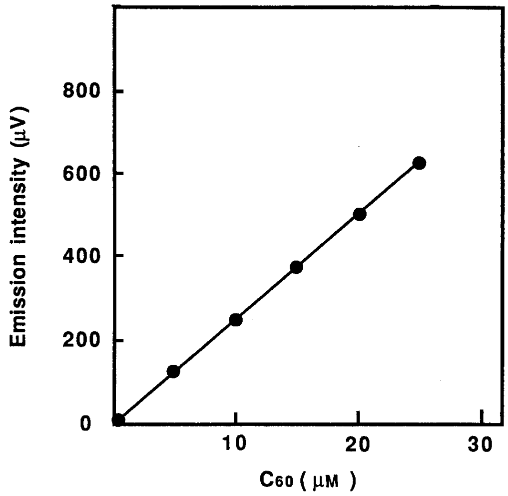

Fig. 4. Effect of C_{60} Concentration on the Singlet Oxygen Emission at 1268 nm from C_{60} Solution

Singlet oxygen generation in C_{60} benzene 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.

Effect of Laser Power and C_{60} Concentration on 1268 nm Emission Intensity Figure 3 shows the relationship between the emission intensity at 1268 nm in C_{60} solution and laser power. The emission intensity of singlet oxygen in laser-excited C_{60} solution increased with the increase of laser power in this range of laser power.

Figure 4 shows the dependence of the emission intensity at 1268 nm on the concentration of C_{60}. The absorbance spectrum of C_{60} at the same concentration (from 5×10^{-6} to 25×10^{-6} M) changed dose-dependently, and the intensity of the absorbance at 514.5 nm was proportional to the concentration of C_{60} (Fig. 5). These results indicate that C_{60} exists as the monomer, and the interaction between molecules is essentially negligible in the benzene

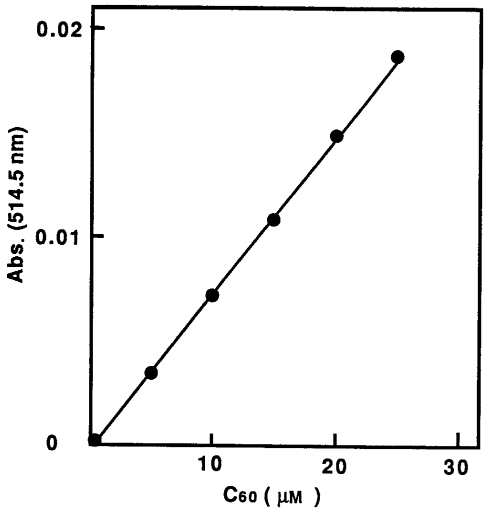

Fig. 5. Relationship between the Absorbance at 514.5 nm and the Concentration of C_{60}

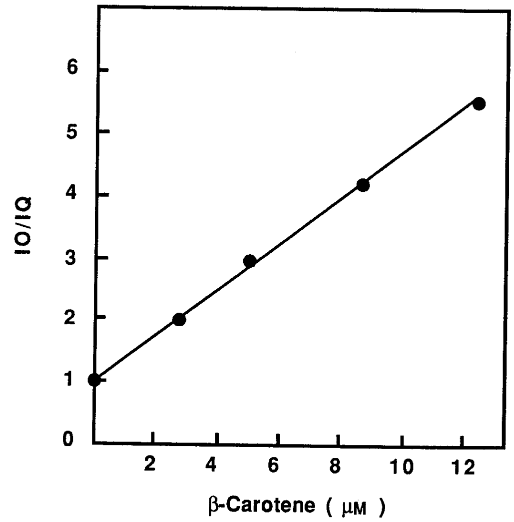

Fig. 6. Stern-Volmer Plots for Quenching of Singlet Oxygen with *β*-Carotene

Singlet oxygen was generated in C_{60} benzene solution excited by Ar laser light at 514.5 nm with 200 mW output power and monitoring was done by measuring the emission intensity at 1268 nm.

solution of C_{60} (from 5×10^{-6} to 25×10^{-6} M).

The emission at 1268 nm was also inhibited by the addition of a singlet oxygen quencher, *β*-carotene. Using C_{60}-excited singlet oxygen generation and quenching of singlet oxygen emission at 1268 nm with *β*-carotene, we determined the rate constant for [*β*-carotene][singlet oxygen]. The Stern–Volmer[11] plot for the ratio of the emission intensity with (IQ) and without (IO) the quencher Q is $IO/IQ = 1 + K_q\tau[Q]$. A plot of IO/IQ *vs.* [Q] is shown in Fig. 6. From the singlet oxygen lifetime in benzene ($\tau = 20$—$30\,\mu s$),[12] the value of $K_q = 1.5$—2.3×10^{10} $M^{-1}s^{-1}$ was obtained for *β*-carotene. This value is in good agreement with that in the literature.[13]

Comparison of Emission Intensity Due to C_{60} and Other Photosensitizers Near-infrared spectroscopic studies in the wavelength region of 1268 nm were performed on other photosensitizer solutions in the same way as described above. The emission of singlet oxygen was detected in MB, RB, and EO benzene–methanol solutions as well. Table I compares the emission intensities of these solutions at 1268 nm. The values were calibrated on the basis of molar extinction coefficient and then normalized with respect to the value of the EO solution. The efficiencies (I/ε) of singlet oxygen generation were determined for MB, RB, EO and C_{60} as 0.1713, 0.0982, 0.0394 and 0.4729, respectively. The efficiency of singlet oxygen production by C_{60} is approximately 12-fold higher than that by EO in benzene–methanol solution under similar conditions. This difference 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 in the lifetime of the triplet state. The triple state of C_{60} may be efficiently quenched by the ground state of dioxygen ($^3O_2$). C_{60} was shown to generate singlet oxygen highly efficiently upon photoirradiation.

Table I. Comparison of Relative Emission Intensities at 1268 nm in Solutions of Various Photosensitizers

Photosensitizer	$A_{514.5}$	$\varepsilon_{514.5}$	O_2 emission (I)	I/ε	I/ε (rel.)
MB	0.0283	2830	485	0.1713	4.3
RB	0.2729	27290	2680	0.0982	2.5
EO	0.5471	54710	2160	0.0394	1.0
C_{60}	0.0074	740	350	0.4729	12.0

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

Discussion

In this work, we firstly measured the direct near-infrared emission spectrum corresponding to the $O_2(^1\Delta_g)\rightarrow O_2(^3\Sigma_g{}^-)$ transition of singlet oxygen in C_{60} solution under laser excitation. The emission dependence on the intensity of laser power and on the concentration of the C_{60} suggests that C_{60} generates singlet oxygen quite efficiently upon photoirradiation, over a wide range of concentration.

We also compared the emission intensity of the C_{60} solution at 1268 nm with those of other dye solutions. The generation of singlet oxygen in C_{60} benzene–methanol solution was found to be 12-fold higher than that of EO benzene–methanol solution, and several-fold higher than with MB and RB (Table I). Although MB, RB and EO are the most frequently used sensitizers in photooxygenation and photodynamic studies, C_{60} had a much higher capacity of singlet oxygen generation than these sensitizers.

Because C_{60} possesses a high degree of symmetry, transitions between the singlet state (S_0) and the singlet state (S_1) are strongly forbidden. This forbiddenness, the small S–T splitting, the very low value of the fluorescence rate constant, and the large spin–orbital interaction in the spherical C_{60} molecule explain why intersystem crossing (ISC) is a dominant process. Therefore, the triplet state of C_{60} is formed in high yield and the triplet life time is very long ($40\pm4\,\mu s$).[9] Generation of singlet oxygen may occur by energy transfer from the highly populated C_{60} triplet state to the dioxygen ground state ($^3O_2$).

The intense absorption peak of C_{60} at 335 nm indicates that C_{60} generates large amounts of singlet oxygen under UV irradiation. Its very high singlet oxygen yield, especially under UV irradition, and the inertness to photooxidative destruction suggest great potential for photodynamic action in biological systems.[14)]

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