Formation of Singlet Oxygen Photosensitized by Aromaic Amino Acids in Aqueous Solutions

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Spectral and kinetic evidences are provided for the formation of singlet oxygen photosensitized by aromatic amino acids in air-saturated water. Irradiation of these solutions at 266 nm induces infrared luminescence with a time-resolved spectrum peaking at 1270 nm and a lifetime characteristic of singlet oxygen. In the cases of tryptophan, tyrosine and phenylalanine, the radiative lifetimes are 3.3 ± 0.1μs, 3.9 ± 0.1 μs and 3.7 ± 0.1μs, respectively.

The excited singlet state of oxygen $^1O_2(^1\Delta_g)$ is involved in the dye-sensitized photooxydation leading to inactivation of various living cells.[1] In this context, the reactions of $^1O_2$ with cell constituents, such as proteins and nucleic acids, have been extensively studied from the perspective of its effect on biological molecules [2] and also photodynamic therapy of malignancies.[3] The $^1O_2$ thus involved in photooxydation is interpreted to be generated through the higher-lying electronic state when an excited triplet dye-molecule reacts with oxygen in solution.[4] Although the interaction of tryptophan and tyrosine with $^1O_2$ can cause the photooxydation,[5-7] these amino acids could also cause the $^1O_2$ formation because the excited triplet states might react with oxygen in solution. To prove this hypothesis, we present evidence for the generation of $^1O_2$ photosensitized by aromatic amino acids tryptophan, tyrosine, and phenylalanine in aqueous solutions.

The singlet oxygen was detected by spectral and kinetic measurements of the 1270 nm phosphorescence that the $^1O_2$ emits in aqueous media. The measurements were carried out using a microsecond photon counting spectrophotometer which allowed us the simultaneous detection of both the spectral- and time-resolved emissions. Our instrument was based on the use of a photomultiplier highly sensitive in the near-IR region. The apparatus and measurements have been described previously.[8] Briefly, the solution was irradiated with a small Q-switched Nd:YAG laser (New Wave Research, Mini/Lase-II; 3.1 mJ/pulse at 266 nm; 6 ns duration; 20 Hz repetition rate). A scattered fraction of the laser output was utilized to count the number of exciting pulses and to provide an optical trigger signal. The solution was made to flow at the desired constant rate through a cell (10 mm x 30 mm x 30 mm) to minimize the photodegradation of the sample. The very weak emission at 1270 nm of $^1O_2$ was collimated at right angles to the laser beam. Then it focused on the variable slit of a Spex 1681 monochromator blazed at 1000 nm after passing through a cut-off filter (Sigma Koki, ITF-100RM) to eliminate higher-order effects from the monochromator. The spectrally analyzed light from the monochromator was focused onto the photocathode of a photomultiplier (Hamamatsu, R5509-41) maintained automatically at - 80 °C by N_2 gas cooled in a liquid N_2 container. The signal after amplification was sent to a time-resolving photon counter (Hamamatsu, C2550-01) under direct control from a PC computer (NEC PC-9821 V10) via application programs. Data analyses such as the biexponential curve fit *etc.* were performed using Igor analysis software from WaveMetrics. Tryptophan, tyrosine and phenylalanine were purchased from Tokyo Kasei Kogyo, Japan, and used as photosensitizers without further purification. The samples were freshly prepared in water (pH 6.2) distilled twice. Some overlapping luminesence of photoproducts related to sensitizers entered the photomultiplier and therefore obscured the spectral profile and the rise of near-IR $^1O_2$ emission. The measurement by time-resolving photon counter offers a variety of improvements in spectroscopic and kinetic studies of singlet oxygen.

Figure 1 (A) demonstrates the time-resolved emission spectrum of 0.1 mM (1 M = 1 mol dm^{-3}) tryptophan in air-saturated aqueous solution. The spectrum was obtained with a time integration in the time window from 1.8 μs to 15 μs after a trigger signal to eliminate the overlapping sensitizer-related luminescence. The monochromator with 5 nm slit widths was set to scan a wavelength region from 1150 nm to 1350 nm at 2.5 nm intervals, *i.e.* 80 wavelength points were taken. Each wavelength point required an acquisition interval of about 1 min, corresponding to 1200 shots of laser pulse. The sample irradiated at 266 nm was made to flow through the cell at a rate of 0.3 ml/s. The spectrum obtained shows the Gaussian-like profile characteristic of the $^1O_2$ emission at around 1270 nm in aqueous solution.[8]

Normalized curves shown by "Air" and "Ar" in Figure 1(B) demonstrate the rise and decay of the 1270 nm emission generated

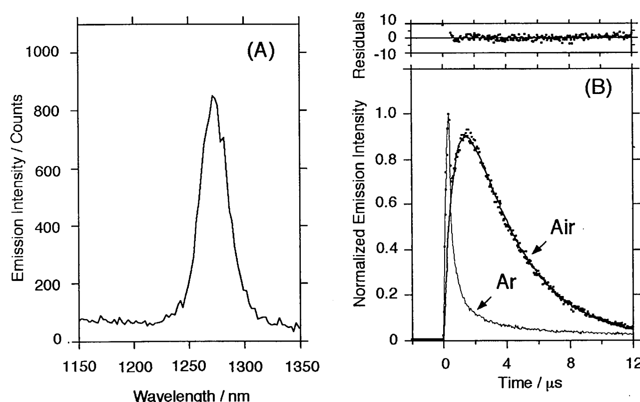

Figure 1. (A) Time-resolved spectrum of infrared emission from air-saturated solution of 0.1 mM tryptophan in water. The spectrum was obtained by applying a time integration in the time window from 1.8 μs to 15 μs after a trigger signal. (B) Normalized kinetic curve and biexponential fit with residuals of 1270 nm emission signal from air-saturated solution of tryptophan in water (Measured points: dots Fitted curve: solid line indicated by "Air"), and the normalized decay from the argon-saturated aqueous solution (Ar).

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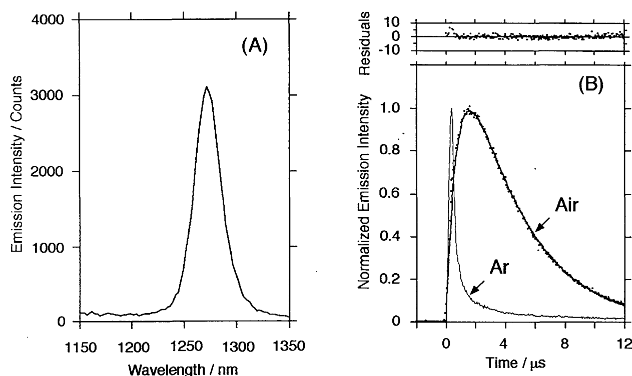

Figure 2. (A) Time-resolved spectrum of infrared emission from air-saturated solution of 0.5 mM tyrosine in water. Time integration: from 3 μs to 18 μs. (B) Normalized kinetic curves of 1270 nm emission from air- and argon-saturated solutions of tyrosine in water. Namings and signs are the same as in Figure. 1.

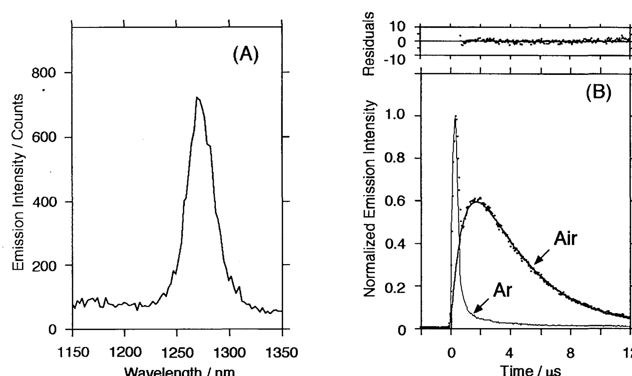

Figure 3. (A) Time-resolved spectrum of infrared emission from air-saturated solution of 5 mM phenylalanine in water. Time integration: from 2 μs to 17 μs. (B) Normalized kinetic curves of 1270 nm emission from air- and argon-saturated solutions of phenylalanine in water. Namings and signs are the same as in Figure. 1.

from respective air- and argon-saturated aqueous solutions of tryptophan. Dots are measured points obtained in the air-saturated aqueous solution. As is seen in Figure 1(B), the kinetic curve for the Ar-saturated solution begins with a sharp spike at the excitation time followed by a rapid decay. The sharp spike is assumed to be the luminescence of photoproducts related to tryptophan. On the other hand, the measured curve shown by dots for the air-saturated solution increases after the spike and then decreases with the elapse of time. The time-resolved emission data were fit to the following biexponential expression:

$$F(t) = A[-\exp(-t/\tau_1) + \exp(-t/\tau_2)] \quad (1)$$

where $F(t)$ is the theoretical function of the $^1O_2$ emission with respect to time, t, and A is the amplitude at a defined wavelength.[7,9,10] τ_1 and τ_2 denote the rise and lifetime of the $^1O_2$ emission, respectively. The solid line shown by "Air" in Figure 1(B) indicates the fitting curve for the $^1O_2$ rise with $\tau_1 = 0.75 \pm 0.01$ μs and the decay with $\tau_2 = 3.3 \pm 0.1$ μs. Calculated residuals indicate that the curve fit converged properly. The lifetime of $\tau_2 = 3.3$ μs is in good agreement with published results in which the lifetime is about 3.09 μs to 4.2 μs under similar measurement conditions.[11,12] Thus, taking into consideration the spectral and kinetic results, it may be said that the singlet oxygen is generated through photosensitization of tryptophan and emits the luminescence at around 1270 nm in aqueous solution.

The time-resolved emission spectra and their kinetic curves of 0.5 mM tyrosine and 5 mM phenylalanine in aqueous media are shown in Figures 2 and 3, respectively, where the namings and signs of data *etc.* are the same as in Figure 1. The measuring conditions are also the same for the sample irradiation of tryptophan. As shown in Figures 2(A) and 3(A), both Gaussian-like spectra peaking at 1270 nm are characteristic of the profile of the $^1O_2$ luminescence. In accordance with these results, the kinetic curves shown in Figures 2(B) and 3(B) suggest that the singlet oxygen is formed by irradiation of tyrosine and phenylalanine in the presence of oxygen because only the fast spikes appear in both Ar-saturated solutions. In the case of tyrosine, the fitting function calculated by eq. (1) gives the $^1O_2$ rise with $\tau_1 = 0.83 \pm 0.01$ μs and the decay with $\tau_2 = 3.9 \pm 0.1$ μs, whereas the biexponential fit of the kinetics of $^1O_2$ generated from phenylalanine yields $\tau_1 = 0.93 \pm 0.01$ μs and $\tau_2 = 3.7 \pm 0.1$ μs, respectively. Calculated residuals indicate that the curve fit converged properly. Differing from one another, the values of lifetime obtained from both solutions are within the range of published values mentioned above. The discrepancies between the lifetimes of $^1O_2$ generated from three amino acids are assumed to be caused by the reaction rate of singlet oxygen produced with respective amino acids. Thus, it may be said that the 266 nm pulsed laser irradiation of air-saturated aqueous solutions of tryptophan, tyrosine and phenylalanine induces near-IR emission of singlet oxygen with a peak at 1270 nm by photosensitization of these aromatic amino acids.

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