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Behavior of Singlet Oxygen in Vitamin E Emulsion

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The decay rate constant of the phosphorescence of singlet oxygen ($^1O_2$) was measured in a vitamin E (VE) emulsion system by single-photon counting. The results suggest that the $^1O_2$ emission mainly occurs around the oil droplets of VE rather than in the bulk solution.

The electronically excited $^1\Delta_g$ state of molecular oxygen is usually denoted as "singlet oxygen" ($^1O_2$), and is recognized as a reactive oxidizing species.[1,2] In general, $^1O_2$ is generated through photosensitization by dye molecules. Accordingly, it sometimes plays an important role in various photochemical and photobiological processes.[1–9] Because $^1O_2$ induces and accelerates oxidation of materials, methods and processes protecting from $^1O_2$ are very important not only in biological systems but also in industrial products. UV-protection and use of antioxidants are very effective for prevention of degradation by $^1O_2$, and are very important in cosmetics for skin care and in medicine since $^1O_2$ generation is thought to induce photodegradation and cancer of the skin.[10] On the other hand, photodynamic therapy using $^1O_2$ generation is hoped to be an effective future medical treatment for destroying malignant tumor or cancer cells.[11] Research on $^1O_2$ has progressed in a wide variety of areas.[1–13]

Vitamin E (VE) is well known as a naturally available antioxidant. The natural VE group includes α-, β-, γ-, and δ-tocopherols, which have a long alkyl-chain and are oily compounds.[14,15] In biological systems, these natural tocopherols are believed to exist and function in hydrophobic environments, such as biomembranes. Many reports suggest significant protective effects of tocopherols against oxidative stress and reactive-oxygen species (ROS).[14,15] These effects are generally explained by the low oxidation potential displayed by this group of molecules. Furthermore, VE is reported to be a very good quencher of $^1O_2$.[1,12] The second-order rate constant of VE for $^1O_2$ quenching is reported to be around $10^8\,M^{-1}\,s^{-1}$, which is a very large value compared with those of the other natural compounds except for carotenoids.[1,3,12]

VE is extensively used as an antioxidant or a medicinally functional additive in foods, drinks, supplements, and drugs, and also used in medicine.[14] Natural VE's are poorly water-soluble, but they can be easily dispersed in water-rich solutions often with the use of surfactants or functional compounds. As a result, VE-rich emulsion solutions can be obtained. Our interest in this study is the behavior of VE-rich oil droplets with respect to the $^1O_2$ dynamics in such emulsion media.

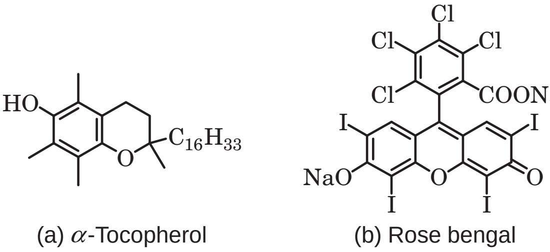
"Emulsion" is an inhomogeneous liquid or cream-like system which is a mixture of two or more liquids, such as water and oil, which do not mix naturally.[16] It is often found in natural or industrial materials, for example, foods, drugs, cosmetics, drinks, paints, and photographic film systems. In typical emulsion systems, numerous small oily droplets are suspended in a water-rich solution. The behavior of $^1O_2$ in emulsion systems is important and of our interest. However, kinetic and quantitative studies on this theme have been difficult because of the interference of opacity and photo-scattering of samples. In order to investigate the $^1O_2$ dynamics and the antioxidant process versus $^1O_2$ in emulsion systems, an accurate method for observing $^1O_2$ is required.[5–9]

In this work, investigating $^1O_2$ behavior in α-tocopherol containing emulsion systems, the measurement of the $^1O_2$ decay rate was carried out by observing $^1O_2$ phosphorescence by single-photon-counting. Detection of the phosphorescence of $^1O_2$ ($^1\Delta_g$) around 1270 nm is the most accurate and reliable method for studying $^1O_2$ dynamics.[4–7] Furthermore, the single-photon-counting method can avoid the occurrence of bimolecular self-quenching among $^1O_2$ by using low photoexcitation power.[4] This method is very useful when applied to opaque and scattering samples, such as the present emulsion systems. From the obtained results, the effect of the inhomogeneous systems on the $^1O_2$ behavior and quenching is discussed.

Experimental

α-Tocopherol (Fig. 1a) and rose bengal (RB, Fig. 1b) were obtained from Wako Pure Chemicals, and were used as received. Ethanol (Wako) was dried and purified by distillation. Deionized water was treated with an ion-exchange column (Millipore Milli-Q). All sample solutions contained RB ($2.0 \times 10^{-4}\,mol\,dm^{-3}$ (M)) as a photosensitizer. The VE emulsion solutions were prepared in 1:1 (v/v) ethanol/water mixed solvent (E/W) by mixing an α-tocopherol and RB ethanol solution with the same amount of deionized water. The critical VE concentration for generating the clouded emulsion was estimated to be 1.5×10^{-4} M. The sample solutions were handled under air-saturated conditions.

$^1O_2$ phosphorescence was measured at room temperature using a time-resolved near-infrared fluorescence spectrophotometer (Hamamatsu C-7990-01) operating in single-photon-counting mode.[13] A Nd-YAG laser (CryLas FTSS355Q, SHG: 532 nm, 14 kHz, max power: 45 mW, FWHM 1 ns) was attenuated by an ND filter and used for photoexcitation. An IR700 sharp-cut filter (Sigma Koki) was additionally used for cutting the emission of RB and the second-order diffraction light.



(a) α-Tocopherol (b) Rose bengal

Fig. 1. Structures of (a) α-tocopherol and (b) rose bengal.

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Laser irradiation of the RB containing sample generates $^1O_2$ ($^1\Delta_g$) through energy transfer (ET) (photosensitization) from the lowest-excited triplet state of RB ($^3RB^*$) to the ground state O_2 ($^3\Sigma_g$) (reaction 1).

$$\begin{aligned} &RB \overset{h\nu}{\to} {}^1RB^* \longrightarrow {}^3RB^* \\ &{}^3RB^* + {}^3O_2 \xrightarrow{ET} RB + {}^1O_2 \\ &{}^1O_2 \overset{k_0}{\to} {}^3O_2 + h\nu\ (\approx 1274\,\text{nm}), \end{aligned} \tag{1}$$

where $^1RB^*$ and k_0 denote the lowest-excited singlet state of RB and the first-order rate constant for the natural decay of $^1O_2$ in the medium, respectively. The rise of the time-profile of the $^1O_2$ emission is usually characterized to the $^1O_2$ generation process. The apparent decay rate-constant (k_d) for the $^1O_2$ emission at 1274 nm was obtained by a least-squares fit of the time decay curve to a single-exponential curve taking the back-ground counts into account. When the $^1O_2$ quenching progresses through the bimolecular process between $^1O_2$ and a quencher (Q) (reaction 2), the $^1O_2$ decay rate-constant is expressed as follows:[13]

$$^1O_2 + Q \overset{k_q}{\to} \text{Quenching products}, \tag{2}$$

$$k_d = k_0 + k_q[Q], \tag{3}$$

where k_q is the second-order rate constant of the $^1O_2$ quenching reaction by Q. We can determine the k_q value from a slope of the plot of k_d versus the concentration of Q ([Q]).

Results and Discussion

The spectrum and time profile at 1274 nm for the $^1O_2$ phosphorescence observed in an ethanol solution of RB are shown in Fig. 2. The natural decay rate of $^1O_2$ in ethanol was determined to be $k_0 = 6.49 \times 10^4\,s^{-1}$, which agrees with the reported value.[4] Addition of VE as a $^1O_2$ quencher to the ethanol solution increased the decay rate constant. Figure 3a shows the plot for the k_d value versus the VE concentration ([VE]) obtained for the ethanol solution. The k_d value increased with an increase of [VE]. A linear relationship was obtained in the plot for k_d vs. [VE], and the slope of the plot gave the second-order quenching rate constant ($k_q = 1.22 \times 10^8\,M^{-1}\,s^{-1}$) for α-tocopherol in ethanol. This k_q value is comparable to the reported rate constant in other solvents,[3] and indicates that VE is a very good quencher of $^1O_2$ in ethanol.

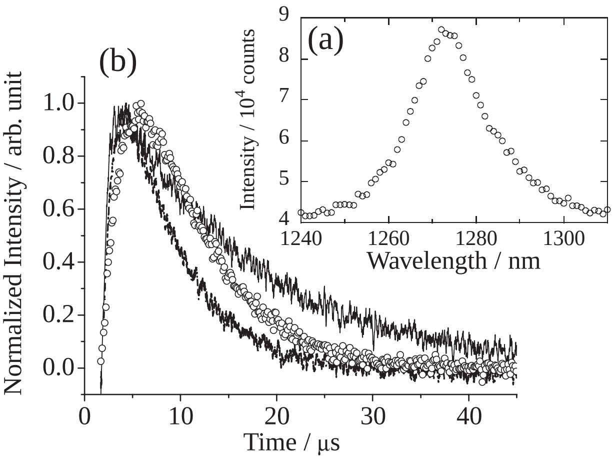

Fig. 2. (a) Emission spectrum of $^1O_2$ in ethanol. (b) Time evolution of $^1O_2$ phosphorescence at 1274 nm in ethanol (solid line), E/W (broken line), and the VE emulsion system (circle). High-frequency random noise was removed by a smoothing method.

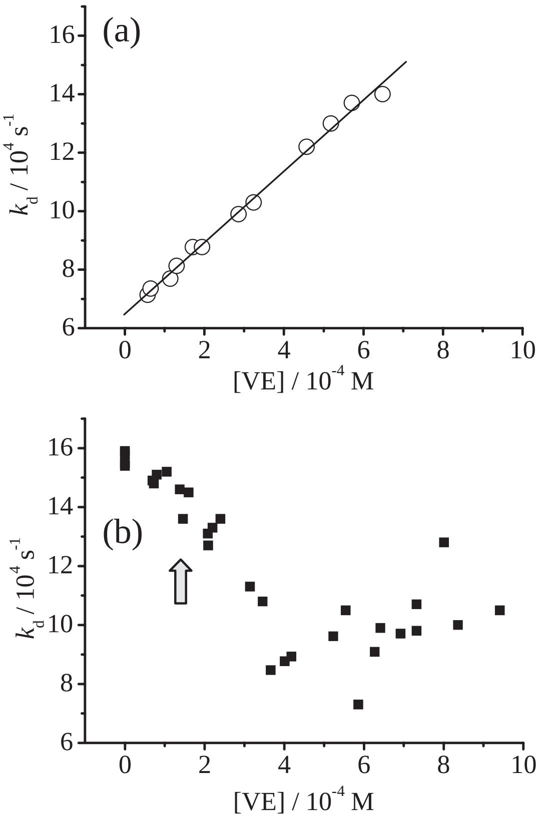

Fig. 3. Plots of k_d vs. [VE] obtained (a) in ethanol and (b) in the E/W solution.

Using a similar method, the decay rate constant of $^1O_2$ was measured for VE emulsion systems with varying [VE]. The plot of k_d vs. [VE] obtained for the E/W solution is shown in Fig. 3b. The time profile at [VE] = 0 (shown as a broken line in Fig. 2b) gave $k_0 = 1.57 \times 10^5\,s^{-1}$ in E/W. This value is 2.5 times as large as that in ethanol and comparable to the mean value ($1.5 \times 10^5\,s^{-1}$) of those in ethanol and water.[4] Addition of over 1.5×10^{-4} M VE (marked by the arrow in Fig. 3b) led to a cloudy E/W solution. The time profile of $^1O_2$ phosphorescence in the emulsion system ([VE] = 3.37×10^{-4} M) is shown as circles in Fig. 2b. The [VE] dependence of k_d in the E/W solution is rather different from that obtained in ethanol. Surprisingly, with an increase of [VE], the k_d value in the E/W solution decreased in [VE] = 1–4 $\times 10^{-4}$ M, and is almost constant ($\approx 9.5 \times 10^4\,s^{-1}$) in [VE] $\geq 5 \times 10^{-4}$ M. The relative intensity of the $^1O_2$ emission was 1.0, 0.37, and 0.27 in ethanol, in E/W, and in the emulsion system ([VE] = 3.31×10^{-4} M), respectively.

It is clear that the [VE] dependence of the k_d value in the E/W solution does not come from the $^1O_2$ quenching process. It should be mainly a result of the environment around $^1O_2$, because the $^1O_2$ lifetime is very sensitive to its surroundings, such as the solvents and coexisting compounds including heavy-atoms.[1–4] $^1O_2$ lifetime is relatively longer in aprotic hydrocarbons, such as hexane and toluene, than in protic solvents, such as water and alcohols. In emulsions, RB is thought to be in the bulk E/W phase because RB is more soluble in water and ethanol than in hydrocarbons. In fact, RB was scarcely dissolved in the VE oil (We could not measure the $^1O_2$ phosphorescence in bulk VE oil.). Hence, the $^1O_2$ generation through photosensitization by $^3RB^*$ should occur in the bulk phase outside the oil droplets. However, the [VE] dependence of k_d suggests that the environment of $^1O_2$ varied with increase of [VE].

It is probable that $^1O_2$ phosphorescence occurs in the interface region between the bulk phase and the oil droplets. It may give rise to the decrease of k_d because the hydrophobic property of the oil droplet should reduce the polarity of the local environment. If this is true, we might observe both fast and slow decay components which are related to the $^1O_2$ dynamics in the E/W bulk phase and that around the oil droplet, respectively. However, it was not possible to fit the decay data in the emulsion to a double-exponential curve. This fact suggests that the slow component related to the dynamics near the VE oil droplet dominates that of the bulk phase. The amount of $^1O_2$ around the oil droplets might be relatively larger than that in the bulk phase, because molecular oxygen is less soluble in water-rich media than in organic solvents.[17]

Although the E/W solution became cloudy at $[VE] \geq 1.5 \times 10^{-4}$ M, the k_d value already started to decrease from $[VE] = 0.7 \times 10^{-4}$ M. This result suggests that the formation of VE droplets already started at that concentration. In this condition, although the formation of the micro-size droplet is optically ineffective, it noticeably influences $^1O_2$ decay dynamics.

On the other hand, the constant k_d value in $[VE] \geq 5 \times 10^{-4}$ M means that the environment around the oil droplet becomes steady beyond that point. Similar behavior was observed for the rate constant of the antioxidant reaction versus DPPH radical.[18] The [VE] dependence of k_d may come from the size of the VE droplet. Droplet size is considered to be distributed in the order of 0.1–1 µm in the present whitely clouded emulsion.[16] In $[VE] = 1–4 \times 10^{-4}$ M, the size of the VE droplet might increase gradually with an increase of [VE]. In $[VE] \geq 5 \times 10^{-4}$ M, the droplet size reached the limit for dispersing metastably. Then, the environment around the oil droplet became steady there. The k_d values are a little scattered in $[VE] \geq 6 \times 10^{-4}$ M. This might be because the emulsion system was unstable under these conditions. Actually, when $[VE] \geq 8 \times 10^{-4}$ M, the emulsion partly started to separate into oil and water-containing phases 1 h after the preparation.

In conclusion, the present study shows the peculiar behavior of $^1O_2$ dynamics in a VE emulsion system. The decay rate of $^1O_2$ phosphorescence decreased with the increase of [VE]. The results suggest that $^1O_2$ emission mainly occurs around the VE oil droplets rather than in the bulk phase. The formation of the oil droplets noticeably reflects the $^1O_2$ decay dynamics.

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