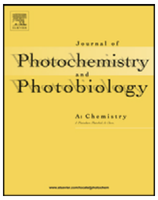
Journal of Photochemistry and Photobiology A: Chemistry 210 (2010) 173–180

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem



Photochemical formation and decay of tocopheroxyl radical in vitamin E emulsion: A laser-photolysis study

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ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form 3 December 2009
Accepted 4 January 2010
Available online 7 January 2010

Keywords:
Vitamin E
Photolysis
Antioxidant
Tocopherol
Emulsion

ABSTRACT

In order to investigate effect of the inhomogeneous dispersion system on photogeneration and decay dynamics of the tocopheroxyl radical (Toc$^•$), transient absorption and steady-state and time-resolved EPR measurements on photolysis of vitamin E (VE) were carried out. Tocopherol (TocH) is photo-decomposed through electron-detachment in the excited state and the subsequent proton releasing, which produce Toc$^•$ and the solvated electron (e^-_{solv}). The decay of Toc$^•$ is explained by (i) a rapid decay within 2 μs by recombination between Toc$^•$ and e^-_{solv}, (ii) a second-order decay observed in the range of 5–150 μs by reaction between Toc$^•$ and the superoxide anion radical ($O_2^{•-}$), and (iii) a slow decay in >10 ms by bimolecular self-quenching of Toc$^•$. The Toc$^•$ decay in the VE emulsion was very different from that in the homogeneous solution. In the emulsion, the Toc$^•$ yield was small, the contribution of the reaction with $O_2^{•-}$ was rather small, and the decay due to the bimolecular reaction of Toc$^•$ was much accelerated. In the emulsion, Toc$^•$ could not be detected by steady-state EPR, although it could in ethanol. These differences should be caused by the oil-droplet formation in the emulsion. From these results, we can expect that the photostability of the VE emulsion is superior to that of the VE homogeneous solutions.

1. Introduction

Emulsion is an inhomogeneous liquid or cream-like system that is often found in natural or industrial materials [1,2]. In typical oil-in-water (o/w) emulsions, numerous micro-oil-droplets are suspending in water. Such emulsion systems are useful and of importance because they can suspend and transfer large amount of lipophilic functional compounds using water-base media. In addition, the emulsion is thought to be a model for biological environment such as liposome, cytoplasm, and serum. Behaviors of emulsion systems containing some antioxidants or pharmacological functional compounds are of great interest and was the subject of our recent studies [3–9]. The authors reported that a stable emulsion dispersing α-tocopherol (α-TocH, Fig. 1a) without surfactants could be made in 1:1 (v/v) ethanol/water mixed solvent (E/W) [6,7]. α-Tocopherol is a lipophilic natural antioxidant known as vitamin E (VE), and is also used widely as a functional additive in many products, such as foods, drinks, supplements, drugs, and cosmetics, as well as in medicine [10–13]. So, the α-TocH containing emulsion is useful for water-base or cream-base products.

In the estimation of antioxidant efficiency or the investigation of reaction kinetics, the emulsion is much complicated because it contains oil-droplets, a bulk phase, and the interfacial region between them. We are interested in behaviors of VE micro-oil-droplet having no protective compound on its surface and of the interface between the oil and the bulk solution phase with respect to the antioxidant action against reactive oxygen species (ROS). Our previous study for singlet-oxygen ($^1O_2$) behavior in the VE emulsion system gave a peculiar result that $^1O_2$ decay rate measured by observing $^1O_2$ phosphorescence at 1274 nm decreased with increase of α-TocH concentration, although α-TocH should work as a good $^1O_2$ quencher [6,7]. This fact suggests that formation of α-TocH droplets and the droplet-size noticeably influence $^1O_2$ decay dynamics, probably because $^1O_2$ is localized near the oil-droplets. On the other hand, in the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, the VE oil-droplet is ineffective against the radical scavenging and only the VE fraction dissolved in the bulk phase essentially scavenges DPPH [14]. Thus, the emulsion often shows different behavior, toward the antioxidant action against ROS, from homogeneous solutions.

It is well-known that tocopherol (TocH) is easily decomposed and leads to the tocopheroxyl radical (Toc$^•$) by ultraviolet (UV) light (UV-B, <310 nm) irradiation as well as many phenol compounds [15–17]. Such photochemical generation of Toc$^•$ by UV is often a serious problem for biological systems containing VE because Toc$^•$ is supposed to initiate and accelerate the lipid-peroxidation [18–21]. At this point, the emulsion system may have a possibility to stabilize large amount of VE because the emulsion strongly scatters UV and some water-soluble additives as stabilizers are available

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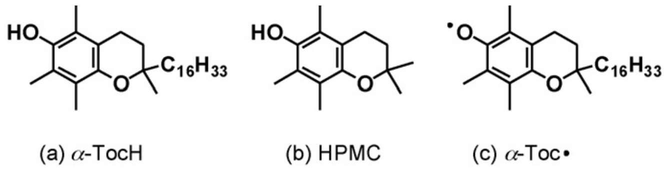
doi:10.1016/j.jphotochem.2010.01.001



(a) α-TocH (b) HPMC (c) α-Toc•

Fig. 1. Molecular structures of α-TocH, HPMC and α-Toc•.

in the aqueous phase. Thus, photochemical generation and decay dynamics of Toc• in the VE emulsion are of interest. In this study in order to investigate the effect of the inhomogeneous dispersion system on generation and decay dynamics of Toc•, transient absorption measurements and steady-state and time-resolved EPR measurements on the photolysis of the VE emulsion system were carried out. The reaction kinetics for Toc• and the advantage of the VE emulsion system as a functional system are discussed.

2. Experimental

α-Tocopherol was obtained from Wako Pure Chemicals, and was used as received. 6-Hydroxy-2,2,5,7,8-pentamethylchroman (HPMC, Fig. 1b) was obtained from Eisai, and was used as a soluble α-tocopherol homologue in 1:1 (v/v) ethanol/water mixed solvent (E/W). Ethanol (Wako) was dried and purified by distillation. The VE emulsions were prepared by mixing an ethanol solution of α-tocopherol and the same volume of deionized water treated with an ion-exchange column (Millipore Milli-Q) [6]. For reducing oxygen dissolved in sample solutions, N_2 gas bubbled for 5 min before transient absorption experiments.

Transient absorption spectra of sample solutions in a 1 cm-square quartz cell were measured at room temperature with a conventional nanosecond laser-photolysis system (UNISOKU TSP-1000), using an Nd-YAG laser (Continuum Surelight-I, FHG 266 nm, FWHM < 5 ns, 2 Hz) as the excitation light source [22]. Additional sharp-cut filters (UV30 or Y50) were used for cutting the laser scattering light and the second-order diffraction light. Time profiles of the transient absorption were obtained by accumulating 4–16 waves with a digital oscilloscope (Sony-Tektronix TDS3032).

Steady-state and time-resolved EPR measurements were carried out at room temperature with a JEOL FA-100 EPR spectrometer [23]. Time-resolved EPR (TR-EPR) spectra were recorded with a boxcar integrator (Stanford Research System SR-250) whose gate width was kept at 0.2 μs at several delay times. An Nd-YAG laser (Continuum Surelight-I, FHG 266 nm, 9.5 Hz) was used for photoexcitation. The sample solution was deoxygenated by bubbling of N_2 gas before and during the experiment, and was flowed through a quartz flat cell (optical path: 0.3 mm) in the EPR cavity.

3. Results and discussion

3.1. Photolysis of VE in homogeneous solutions

Fig. 2a shows time development of the transient absorption spectrum observed in photolysis of α-TocH (4.00×10^{-4} M) in ethanol. The spectrum which has absorption maxima at 425, 410, and ∼330 nm is assigned to be the spectrum due to the α-tocopheroxyl radical (α-Toc•, Fig. 1c) [15,17,24,25]. The α-Toc• spectrum decayed with time, but could be observed still at the delay time of more than 300 ms. The rise of the α-Toc• absorption was within 15 ns from the laser pulse. The 425 nm absorbance at the delay time of 1 μs was almost proportional to the excitation laser light intensity. The result of the laser power dependence indicates that the photoreaction producing α-Toc• is a monophotonic process. The experiment under O_2-saturated conditions also gave a similar α-Toc• spectrum. These results suggest that the α-Toc• generating reaction progresses from the singlet excited state.

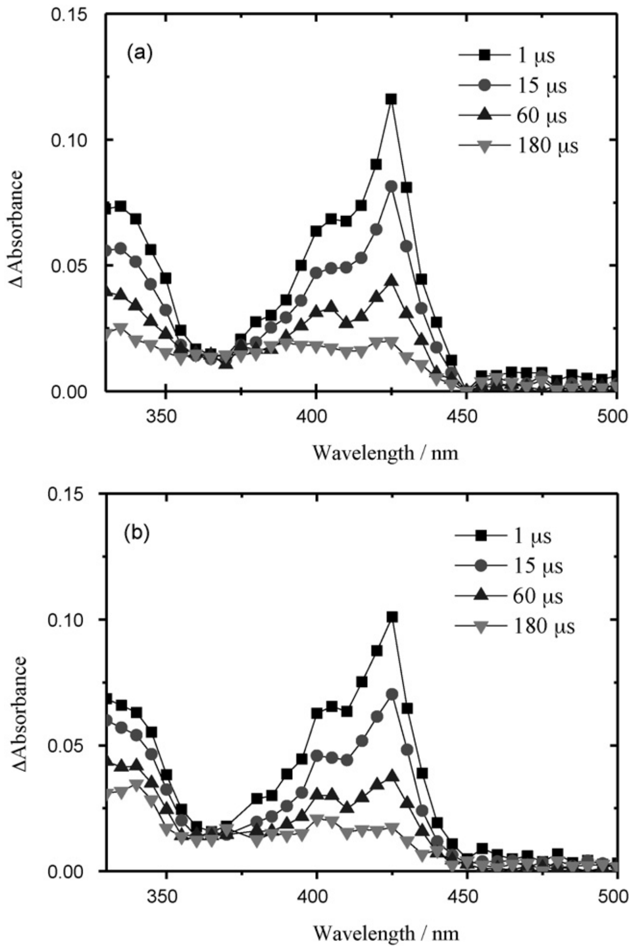


Fig. 2. Time evolution of the transient absorption spectrum in the photolysis of (a) α-TocH (4.00×10^{-4} M) and (b) HPMC (4.04×10^{-4} M) in ethanol.

In the literature, the photodecomposition of TocH is represented by reaction (1), that is, the electron-detachment of TocH in the excited state and the subsequent proton (H^+) releasing, which produce Toc• and the solvated electron (e^-_{solv}) [15]. This photodecomposition of TocH was reported to occur as a monophotonic process through the singlet excited state (^{1}TocH*) [15]. These facts are consistent with the present result.

$$\mathrm{TocH} + h\upsilon \rightarrow {}^1\mathrm{TocH}^* \rightarrow \mathrm{Toc}^\bullet + e^-_{\mathrm{solv}} + \mathrm{H}^+ \qquad (1)$$

The broad absorption band at 550–700 nm was observed only at the delay time less than 2 μs [15]. The decay curve of this band (Fig. 3a) was approximately a single-exponential curve whose time-constant was 0.61 μs (at 620 nm). It should come from e^-_{solv} [15]. This absorption band due to e^-_{solv} disappeared completely under air-saturated conditions, indicating that O_2 quenched e^-_{solv} immediately (reaction (2)) [15,26]. Transient absorption bands due to species other than α-Toc• and e^-_{solv} were negligible in the range of 320–700 nm.

$$e^-_{\mathrm{solv}} + \mathrm{O}_2 \rightarrow \mathrm{O}_2{}^{\bullet-} \qquad (2)$$

Fig. 3b–d shows time profiles of the transient absorption at 425 nm due to α-Toc• observed in the photolysis of α-TocH (4.00×10^{-4} M) in ethanol. The decay curve for α-Toc• consists of at least three components having different time-constants, i.e., (i) a rapid decay within 2 μs, (ii) a decay observed in the range of 5–150 μs, and (iii) a slow decay observed in the range larger

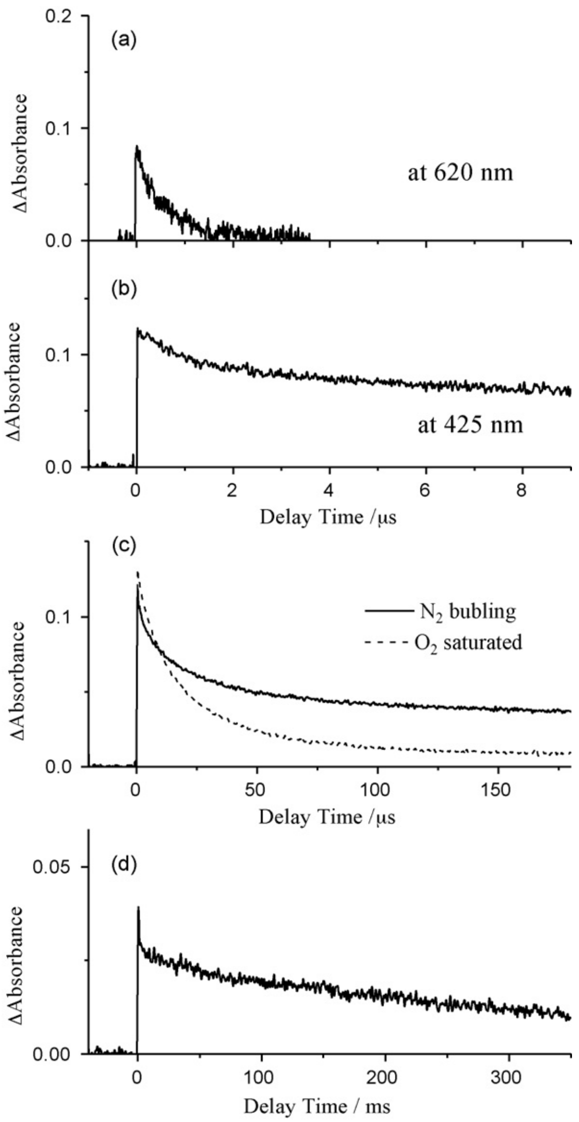


Fig. 3. Time profiles of the transient absorption at 620 and 425 nm in the photolysis of α-TocH in ethanol. High-frequency random-noise was removed by a smoothing method.

than 100 ms. As shown in Fig. 3b, there is 20–30% contribution of the rapid decay component characterized approximately as a single-exponential curve whose time-constant is 0.64 μs. Since the time-constant of the rapid decay component of α-Toc• (0.64 μs) is very close to the lifetime of e^-_{solv} (0.61 μs), the rapid decay component is explained by recombination process between α-Toc• and e^-_{solv} and the subsequent H⁺ capture (reaction (3)). Since considerable part of e^-_{solv} quenched by O_2 (reaction (2)) as a pseudo-first order process, e^-_{solv} showed approximately a single-exponential decay (Fig. 3a). As a result, the rapid decay of Toc• caused by reaction (3) also showed a single-exponential behavior.

$$\text{Toc}^\bullet + e^-_{\text{solv}} + \text{Toc}^-$$

$$\text{Toc}^- + \text{H}^+ \rightarrow \text{TocH} \qquad (3)$$

On the other hand, the slowest decay component whose time-constant is apparently ~0.6 s (Fig. 3d) is thought to be a second-order decay mainly due to bimolecular self-quenching of α-Toc• (reaction (4)) [20,24,27]. It is known that the α-Toc• self-quenching is caused by the dimerization or disproportionation reaction, and the self-quenching rate constant in ethanol was reported as $k_{\text{sq}} = 1.3 \times 10^3\ \text{M}^{-1}\,\text{s}^{-1}$ [24]. Thus, α-Toc• can be observed even after some seconds from its generation in ethanol. However, decay rates in this time region (>100 ms) cannot be determined accurately with our transient absorption instrument because apparent decrease of transient species by diffusion from the measurement area of the cell is not negligible.

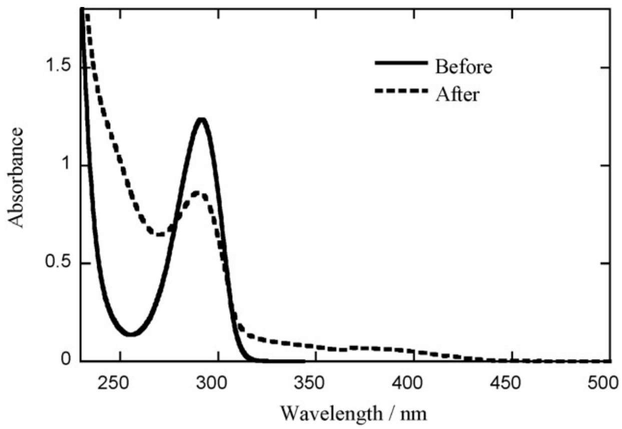


Fig. 4. Absorption spectra of α-TocH (4.00×10^{-4} M) in ethanol before and after the photoirradiation (266 nm, 9.5 Hz, 5 min).

$$\text{Toc}^\bullet + \text{Toc}^\bullet \rightarrow (\text{Colored products}) \qquad (4)$$

Fig. 4 shows the steady-state absorption spectrum of α-TocH (4.00×10^{-4} M) in ethanol before and after the laser irradiation (266 nm, 2 Hz, 5 min). New broad band absorption at 240–450 nm appeared after the photoirradiation. This band is thought to be due to a yellow-brown colored product from α-TocH, such as α-tocopherolquinone which is known as an efficient initiator of photodegradations of lipids [24]. The absorption band at 330–440 nm in the transient absorption spectrum at the later time region (Fig. 2) is explained by the contribution of this colored product.

The decay component observed in the time range of 5–150 μs (Fig. 3c) can be characterized as a second-order decay curve. However, it cannot be explained by the ordinary bimolecular self-quenching of α-Toc• described above. Large increase in contribution of this decay component was observed under O_2-saturated conditions, and this decay component was reported to disappear under completely deoxygenated conditions [15]. These facts suggest that this decay component comes from a reaction between α-Toc• and a short-lived reactive oxygen species. It was reported that the superoxide anion radical ($O_2^{\bullet-}$) was produced by the rapid reaction between e^-_{solv} and O_2 (reaction (2)) [15,26,28]. So, in the early time region (<2 μs), e^-_{solv} is thought to be quenched both by O_2 and by Toc• (reactions (2) and (3)). In view of the fact that the same amount of Toc• and e^-_{solv} should be generated through reaction (1), the contribution of reaction (2) in quenching of e^-_{solv} could be roughly estimated to be 70–80% from the contribution of the rapid decay component due to reaction (3) in the overall Toc• decay. Assuming that reaction (2) occurs in a rate of the diffusion-controlled limit ($k_{\text{dif}} = 9.2 \times 10^9\ \text{M}^{-1}\,\text{s}^{-1}$ at 25 °C [29]) and its contribution in the e^-_{solv} quenching is 75%, the O_2 concentration in the solution after N_2 bubbling is roughly estimated as $[O_2] = 1.3 \times 10^{-4}$ M from the lifetime of e^-_{solv}. This is ~6% of the $[O_2]$ value in ethanol under 1 atm air-saturated conditions (2.07×10^{-3} M) [29]. Because the reaction between α-Toc• and $O_2^{\bullet-}$ (reaction (5)) in organic solvents is known to be much faster ($\geq 10^9\ \text{M}^{-1}\,\text{s}^{-1}$) than the reaction between $O_2^{\bullet-}$ and α-TocH, reaction (5) might play a dominant role in the $O_2^{\bullet-}$ quenching [15].

$$\text{Toc}^\bullet + O_2^{\bullet-} + \text{H}^+ \rightarrow \text{TocH} + O_2 \qquad (5)$$

The Toc$^\bullet$ decay caused by reaction (5) should be treated by a second-order rate equation. Assuming that any reaction other than reaction (5) can be neglected in this time region, the rate equation for the concentration of Toc$^\bullet$ ([Toc$^\bullet$]) is represented by,

$$\frac{d[\mathrm{Toc}^\bullet]}{\mathrm{dt}} = -k_5[\mathrm{Toc}^\bullet][\mathrm{O}_2^-{}^\bullet]. \tag{6}$$

Here, k_5 is the second-order rate constant for reaction (5), and [$\mathrm{O_2}^{\bullet-}$] is the concentration of $\mathrm{O_2}^{\bullet-}$. During this time region, the same amounts of Toc$^\bullet$ and $\mathrm{O_2}^{\bullet-}$ are reduced only by reaction (5), and the initial concentration of $\mathrm{O_2}^{\bullet-}$ ([$\mathrm{O_2}^{\bullet-}$]$_0$) is expected to be smaller than that of Toc$^\bullet$ ([Toc$^\bullet$]$_0$), because $\mathrm{O_2}^{\bullet-}$ should be produced from $\mathrm{O_2}$ and e^-_{solv} by reaction (2) in the earlier stage, but e^-_{solv} may be quenched by some processes in addition to reactions (2) and (3). The integrated rate equation for Eq. (6) is represented as follows.

$$-k_5 t = \frac{1}{\Delta}\ \ln\frac{[\mathrm{Toc}^\bullet]_0[\mathrm{O}_2^-{}^\bullet]}{[\mathrm{O}_2^-{}^\bullet]_0[\mathrm{Toc}^\bullet]} \tag{7}$$

Here, $\Delta = [\mathrm{Toc}^\bullet]_0 - [\mathrm{O_2}^{\bullet-}]_0$ ($\Delta > 0$). In this condition, [$\mathrm{O_2}^{\bullet-}$] is equal to [Toc$^\bullet$] – Δ. Finally, the time development of [Toc$^\bullet$] can be represented by the following equation.

$$[\mathrm{Toc}^\bullet] = \frac{\Delta}{1-f\ \exp(-\Delta k_5 t)} \tag{8}$$

Here, $f = [\mathrm{O_2}^{\bullet-}]_0/[\mathrm{Toc}^\bullet]_0$. The values of [Toc$^\bullet$]$_0$ and [$\mathrm{O_2}^{\bullet-}$]$_0$ can be calculated from the Δ and f values. A least-squares fit of the time-profile data of absorbance at 425 nm (Fig. 3c) to Eq. (8) was successful ($R^2 > 0.999$), and gave the values: $k_5 = 2.19 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$, $\Delta = 8.71 \times 10^{-6}$ M, and $f = 0.632$. Here, $\varepsilon = 4.28 \times 10^3\ \mathrm{M^{-1}\ cm^{-1}}$ was used as the molar absorption coefficient of α-Toc$^\bullet$ at 425 nm according to the literature [24]. The k_5 value obtained is close to the diffusion-controlled limit in ethanol ($k_{\mathrm{dif}} = 9.2 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$ at 25 °C [29]), and is comparable with those reported [15]. The Δ and f values indicate that the contribution of reaction (2) was large in the e^-_{solv} quenching processes.

HPMC is an α-TocH homologue having a methyl group in place of the phytyl-chain of α-TocH at 2-position. The transient absorption result for photolysis of HPMC (4.04×10^{-4} M) in ethanol closely resembled that for α-TocH. The photoreaction processes of HPMC can be explained by a similar way to those for α-TocH (reaction (1)–(5)). The absorption band around 420 nm (shown in Fig. 2b) should be due to the HPMC Toc$^\bullet$ radical. The broad absorption band around 620 nm due to e^-_{solv} was observed at the delay time less than 2 μs, and it disappeared under air-saturated conditions. Fig. 5 shows the time profiles of the transient absorption at 620 and 425 nm. The decay curve for the 425 nm absorption due to Toc$^\bullet$ has three components having different time-constants. Since time-constant of the rapid decay component of Toc$^\bullet$ (0.66 μs) is very close to that of e^-_{solv} (0.62 μs), the rapid decay component is explained by recombination of Toc$^\bullet$ and e^-_{solv} (reaction (3)). The slowest decay component (Fig. 5d) is thought to be the second-order decay due to the bimolecular process of Toc$^\bullet$ (reaction (4)). The decay component in the time range of 5–150 μs (Fig. 5c) can be characterized as a second-order decay coming from the reaction between Toc$^\bullet$ and $\mathrm{O_2}^{\bullet-}$ (reaction (5)). A least-squares fit of the time-profile data of absorbance at 425 nm to Eq. (8) was also successful, and gave the values: $k_5 = 1.56 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$, $\Delta = 7.75 \times 10^{-6}$ M, and $f = 0.67$, when assuming $\varepsilon = 4.28 \times 10^3\ \mathrm{M^{-1}\ cm^{-1}}$. These values are close to those for α-TocH (Table 1). Because HPMC has rather larger solubility in E/W than α-TocH, its homogeneous solution can be obtained even at the concentration of [HPMC] = 4.0×10^{-4} M in E/W. Consequently, HPMC can be used for examining difference between ethanol and E/W in the Toc$^\bullet$ kinetics.

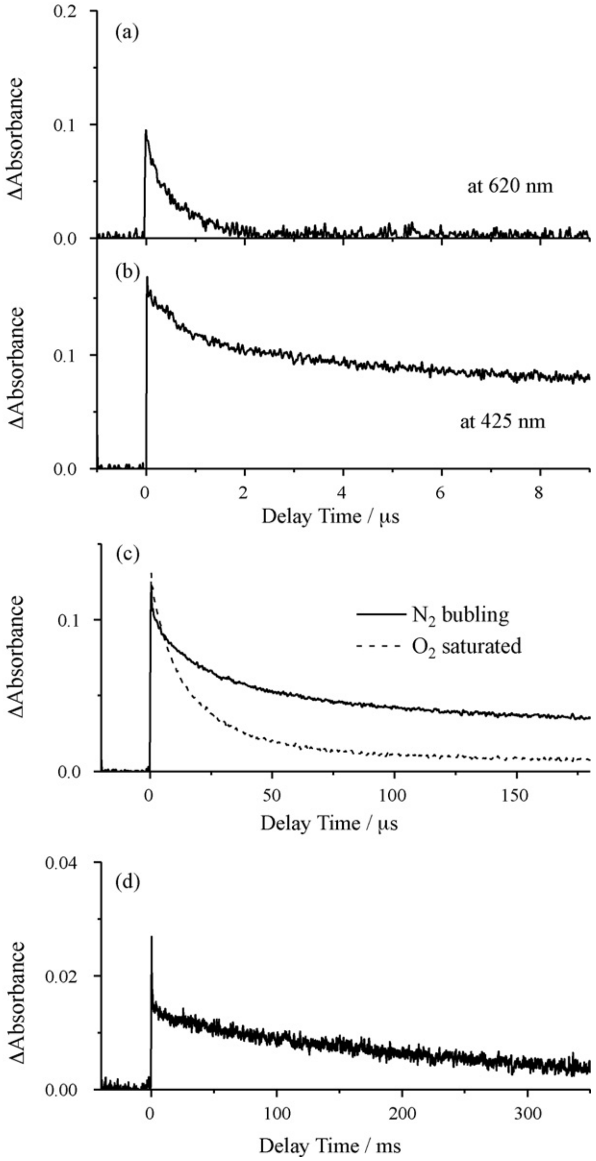


Fig. 5. Time profiles of the transient absorption at 620 and 425 nm in the photolysis of HPMC in ethanol.

Table 1

Values of e^-_{solv} lifetime, time-constant of rapid decay component for Toc$^\bullet$ (τ_3), and k_5 in ethanol and in E/W.

Solvent	Ethanol		E/W	
TocH	α-Tocopherol	HPMC	α-Tocopherol (emulsion)	HPMC
e^-_{solv} lifetime, μs	0.61	0.62	0.021	0.58
τ_3, μs	0.64	0.66	0.020	0.57
k_5, $\times 10^9\ \mathrm{M^{-1}\ s^{-1}}$	2.19	1.56	(2.27)[a]	3.24

[a] A value obtained under $\mathrm{O_2}$-saturated conditions.

The transient absorption result obtained for an E/W solution of HPMC (4.04×10^{-4} M) closely resembled that of the ethanol solution. The photoreaction processes of HPMC in E/W can be explained by a similar way to those in ethanol. The absorption around 420 nm (Fig. 6b) due to Toc$^\bullet$ and the broad absorption band around 620 nm due to e^-_{solv} were also observed. The absorption due to e^-_{solv} disappeared under air-saturated conditions. The absorbance around 425 nm due to Toc$^\bullet$ at the delay time of 1 μs was about a half of that obtained in ethanol, that is, the Toc$^\bullet$ yield in E/W was a half of that in ethanol. This reduction in Toc$^\bullet$ yield indicates decrease

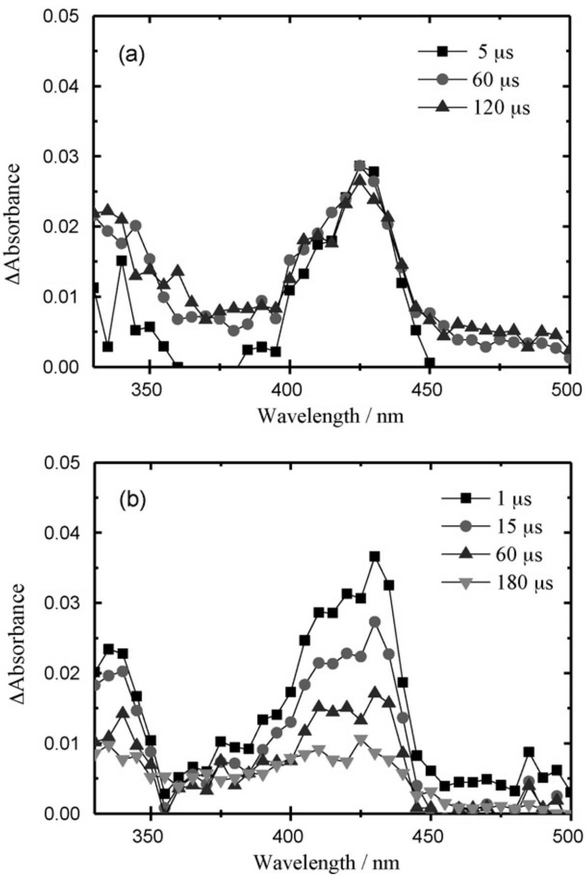


Fig. 6. Time evolution of the transient absorption spectrum in the photolysis of (a) an α-TocH emulsion (4.00×10^{-4} M) and (b) a HPMC solution (4.04×10^{-4} M) in E/W.

in ion-formation efficiency of the excited TocH in E/W. This phenomenon is thought to come from that the reorganization energy and/or the activation Gibbs energy for the transition state might be larger in E/W than in ethanol. Fig. 7 shows time profiles of the transient absorption at 620 and 425 nm. The feature of the time profile is very similar to that in the ethanol solutions of α-TocH and HPMC. The decay curve for Toc• (at 425 nm) consists of three components having different decay time-constants. Since time-constant of the rapid decay component of Toc• (0.57 μs) is almost equal to the lifetime of e^{-}_{solv} derived from the time profile at 620 nm (0.58 μs), the rapid decay is due to recombination of Toc• and e^{-}_{solv} (reaction (3)). The lifetime of e^{-}_{solv} was a little shorter than that in ethanol. The decay component in the time range of 5–150 μs (Fig. 7c) should come from the reaction between Toc• and $O_2^{\bullet -}$ (reaction (5)). A least-squares fit of the data to Eq. (8) gave the values: $k_5 = 3.24 \times 10^9\ \text{M}^{-1}\,\text{s}^{-1}$, $\Delta = 4.05 \times 10^{-6}$ M, and $f = 0.609$ for reaction (5). The k_5 value in E/W is larger than that in ethanol. The result is partially explained by lower viscosity of E/W than that of ethanol. The slowest decay in the time range of >100 ms should be due to the bimolecular self-quenching process of Toc• (reaction (4)). Its time-constant is ~0.3 s, and is comparable to that in ethanol (~0.4 s). The Toc• decay in E/W is not very different from that in ethanol.

3.2. Photolysis of the VE emulsion

As reported previously, a whitely clouded emulsion was obtained in E/W at the concentration of [α-TocH] ≥ 1.5×10^{-4} M (critical concentration for generating an emulsion in E/W) [6,7]. At [α-TocH] ≥ 8.0×10^{-4} M, the emulsion became unstable and partly started to separate into oil and E/W phase. In this study, an emulsion solution of α-TocH in E/W ([α-TocH] = 4.00×10^{-4} M) was used. The α-TocH droplet diameter in this emulsion measured by dynamic-light-scattering (DLS) method was distributed around 0.46 μm [7]. Fig. 6a shows time development of the transient absorption spectrum observed in photolysis of the α-TocH (4.00×10^{-4} M) emulsion in E/W. Because of the light-scattering, the transmittance of the emulsion at 425 nm was 80% (optical path of probe-light: 1 cm), and that at 266 nm through the measurement area (2 mm) irradiated by the excitation laser in the cell was c.a. 40%. The transient absorption spectrum for the α-TocH emulsion resembled that obtained for the VE ethanol solution (Fig. 2a). The rise of the absorption band around 420 nm due to α-Toc• was within 15 ns. The absorbance around 425 nm due to α-Toc• at the delay time of 1 μs was less than a half of that obtained in ethanol. This large decrease of the α-Toc• yield in the emulsion system might be explained by the solvent effect and the light-scattering of the clouded emulsion. As described above, the Toc• yield for HPMC in E/W was a half of that in ethanol. The 425 nm absorbance at the delay time of 1 μs was almost proportional to the excitation

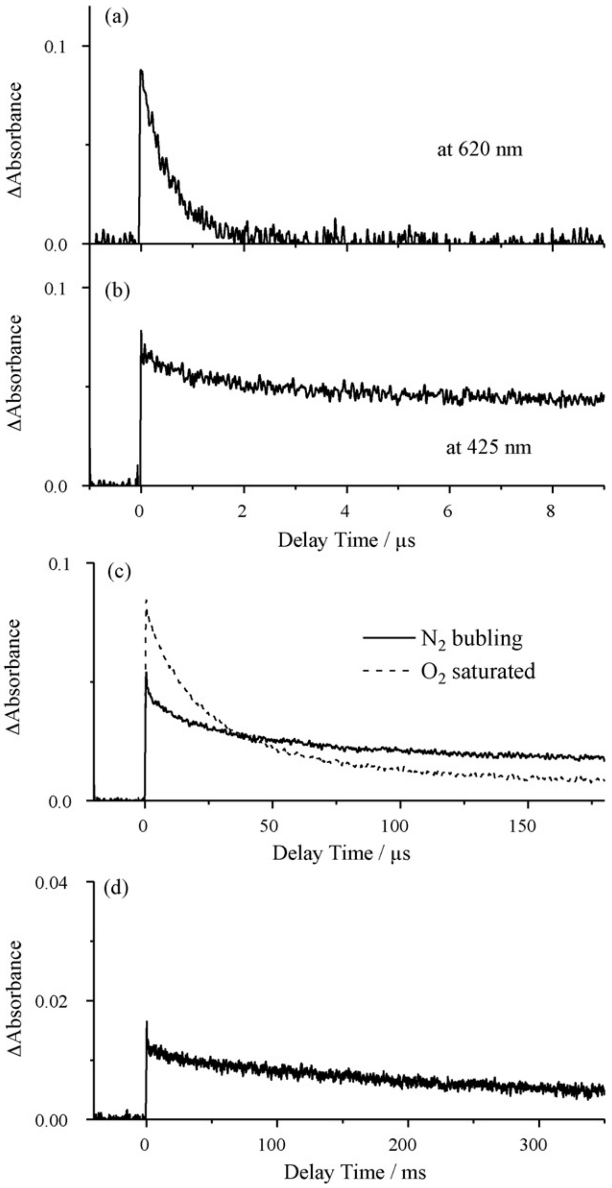


Fig. 7. Time profiles of the transient absorption at 620 and 425 nm in the photolysis of HPMC in E/W.

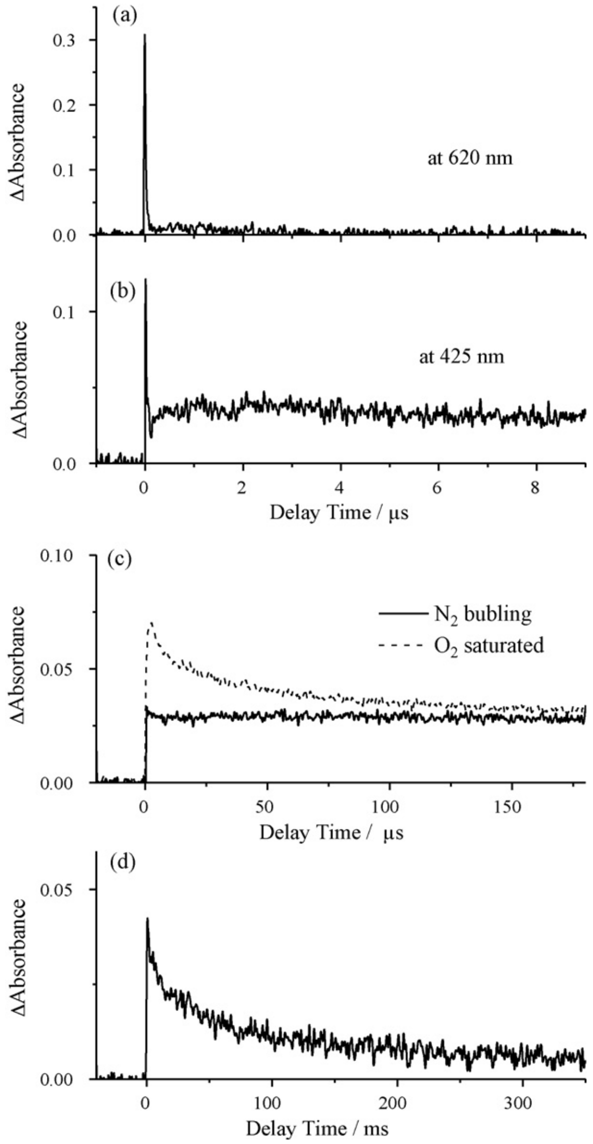


Fig. 8. Time profiles of the transient absorption at 620 and 425 nm in the photolysis of the α-TocH emulsion in E/W.

laser light intensity, indicating that the photoreaction occurred as a monophotonic process. The broad absorption band at 550–700 nm due to e^-_{solv} was observed and it disappeared under air-saturated conditions.

Thus, the α-Toc• generation process in the emulsion is close to that in the homogeneous solution. However, the yields and decay behaviors of α-Toc• and e^-_{solv} in the emulsion are rather different from those in ethanol. Fig. 8 shows time profiles of the transient absorption at 620 and 425 nm obtained for the α-TocH emulsion in E/W. The time-constant of e^-_{solv} decay at 620 nm (Fig. 8a) was only 21 ns, which is significantly shorter than that in the ethanol solution (0.61 μs). In homogeneous solution, the e^-_{solv} decay is caused by the reactions with O_2 and with Toc• (reactions (2) and (3)). However, the O_2 concentration in E/W should be smaller than that in ethanol because O_2 solubility in water is 1/8 of that in ethanol [29]. Therefore, there should be a rapid quenching process for e^-_{solv} other than reactions (2) and (3) in the emulsion. The mechanism for this rapid quenching is not clear, but it should be a result of the oil-droplet formation. The VE droplets might capture and quench e^-_{solv}.

The decay curve for α-Toc• in the N_2 bubbling emulsion sample roughly consisted of two components. One is a rapid decay component whose time-constant is ∼20 ns (Fig. 8b), and another is a slow decay component whose time-constant is ∼70 ms (Fig. 8d). Although the time-constant is much smaller than that in ethanol described above, the rapid decay component is explained by the recombination of Toc• and e^-_{solv} (reaction (3)) because the time-constant is very close to the lifetime of e^-_{solv}. This rapid Toc• decay was caused by the rapid e^-_{solv} decay. In the N_2 bubbling sample, the contribution of the decay component coming from the reaction between Toc• and $O_2{}^{\bullet-}$ (reaction (5)) in the time range of 5–150 μs was negligibly small (Fig. 8c), but it clearly appeared under O_2-saturated conditions. The k_5 value ($2.27 \times 10^9\,M^{-1}\,s^{-1}$) in the O_2-saturated emulsion was as large as that in the ethanol solution. Therefore, the reaction between Toc• and $O_2{}^{\bullet-}$ is thought to occur not in the oil phase but in the bulk E/W phase and/or in the interface region around droplets. The small contribution of reaction (5) in the emulsion is a result of small $[O_2{}^{\bullet-}]$ coming from both the low O_2 concentration and the significantly short lifetime of e^-_{solv}. Interestingly, the slowest decay component (Fig. 8d) which should be due to the bimolecular self-quenching of α-Toc• (reaction (4)) was much accelerated from those in the homogeneous solutions. The time-constant was ∼70 ms in the emulsion while that in ethanol was ∼600 ms. Assuming the second-order decay by reaction (4), the rate constant was estimated to be $2.05 \times 10^6\,M^{-1}\,s^{-1}$, which was significantly larger than that in ethanol [24]. This large acceleration should be caused by the oil-droplet formation, and is explained as follows. Lipophilic α-Toc• may be collected near the hydrophobic oil-droplet. The reaction among α-Toc• occurs in the interface region around the oil-droplet, and is accelerated by reason of the large local concentration of α-Toc•.

The large difference in Toc• decay between the VE emulsion and the homogeneous solution is summarized as follows. (i) The Toc• yield in the VE emulsion was smaller than that in the homogeneous solutions. This is thought to come from the reduction of the ion-formation efficiency of the excited TocH in E/W and the UV light-scattering of the emulsion. This effect may largely suppress the photodegradation of TocH. (ii) The contribution of reaction (3) in the VE emulsion was rather smaller than that in the homogeneous solutions. A rapid quenching process of e^-_{solv} other than reactions (2) and (3) reduces the $O_2{}^{\bullet-}$ yield in the VE emulsion. As a result, the contribution of reaction (5), which is the quenching reaction between $O_2{}^{\bullet-}$ and Toc•, decreases significantly. (iii) The decay component due to the bimolecular termination reaction of Toc• (reaction (4)) in the emulsion was much faster than that in the homogeneous solutions. Lipophilic α-Toc• may be collected near the hydrophobic oil-droplet, and the reaction among α-Toc• occurs in the interface region around the oil-droplet. As a result, the bimolecular reaction of α-Toc• is accelerated by reason of large local concentration of α-Toc•. Evidently, all these differences should be caused by the oil-droplet formation in the emulsion. Especially, the acceleration of the termination reaction of Toc• (reaction (4)) is thought to be very important. The generation and decay behaviors of Toc• in the emulsion are expected to contribute to the photostability of TocH against UV largely.

3.3. Steady-state and time-resolved EPR spectra

Fig. 9a shows a steady-state EPR spectrum obtained during the photolysis of α-TocH (4.00×10^{-4} M) in ethanol by irradiating 266 nm laser with 9.5 Hz repetition. The spectrum is very similar to the reported spectrum [25,30,31], which was assigned to α-Toc• ($g = 2.0046$, A_H (3 equivalent protons) = 0.598 mT, A_H (3) = 0.457 mT, A_H (2) = 0.147 mT, A_H (3) = 0.094 mT). No peak due to the other paramagnetic species could be observed in the spectrum. The EPR signals due to α-Toc• immediately vanished when stopping laser irradiation. The EPR result clearly indicates that α-Toc• is generated through the photoreaction of α-TocH and decays within a few

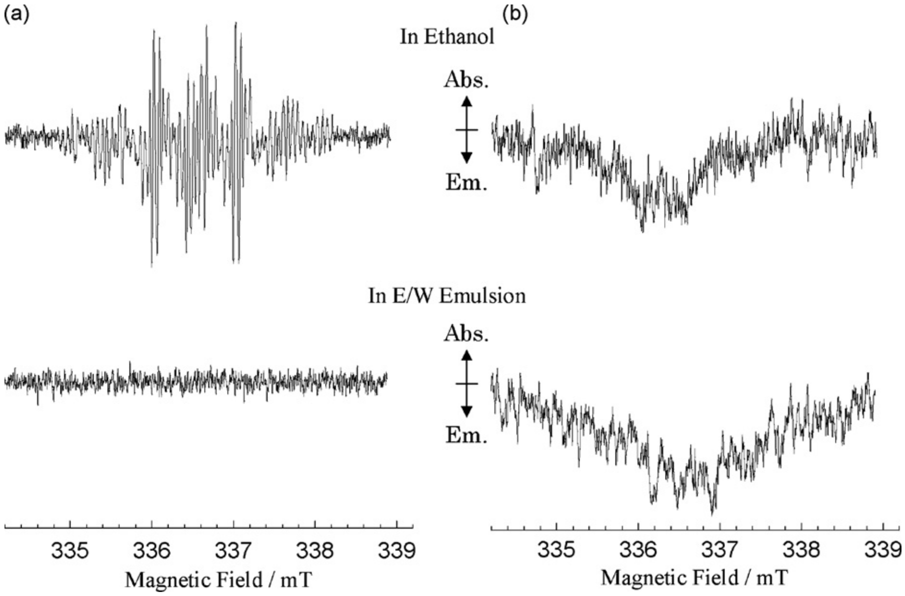


Fig. 9. (a) Steady-state EPR spectra observed for the photolysis of α-TocH in ethanol (top) and in E/W emulsion (bottom). (b) Time-resolved EPR spectra at the delay time of 0.8 μs observed for the photolysis of α-TocH in ethanol (top) and in E/W emulsion (bottom).

seconds in ethanol. The EPR signal intensity of α-Toc• was almost proportional to the excitation laser power, indicating that the photoproduction of α-Toc• progresses as a monophotonic process.

On the other hand, in the steady-state EPR measurement of an α-TocH (1.50×10^{-4} M) emulsion system by irradiating 266 nm laser with 9.5 Hz repetition, no paramagnetic species could be detected (Fig. 9a bottom), although α-Toc• was observed in the transient absorption. Similar results were obtained for the other α-TocH emulsion systems ([α-TocH] = 4.00×10^{-4} M in E/W = 1:1 and 1:3). The result is explained in the following way. In the emulsion systems, because of the short lifetime (~70 ms) and the small yield as described above, the time-averaged concentration of α-Toc• is so small that α-Toc• is not detected by steady-state EPR by irradiating 9.5 Hz laser.

The TR-EPR spectra observed at the delay time of 0.8 μs after the laser pulse in an ethanol solution ([α-TocH] = 4.00×10^{-4} M) and in an emulsion system ([α-TocH] = 1.50×10^{-4} M) are shown in Fig. 9b. Although these TR-EPR spectra are weak, broadened, and noisy, they are thought to be the EPR spectrum of α-Toc•. The TR-EPR signals due to α-Toc• disappeared at the delay time larger than 2.0 μs, while the lifetime of α-Toc• should be much longer. The fact suggests that the observed EPR signals come from the spin polarization due to a CIDEP (Chemically Induced Dynamic Electron Polarization) phenomenon [32–34]. The EPR signal for e^-_{solv} ($g_e \sim 2.000$) could not be detected at the delay time from 0.1 to 2.0 μs.

The TR-EPR spectra show a net emission with a slight *E*/*A* (the low-field side is emissive and the high-field side absorptive) distortion. It is difficult to give a clear and suitable explanation on the CIDEP generating mechanism in this system. In general, the net emissive polarization is explained on the basis of the triplet mechanism (TM) and/or the radical-triplet pair mechanism (RTPM) of CIDEP [23,33,34]. Actually, there are some TR-EPR reports on the photoionization from the highly excited triplet state generated by a biphotonic process [35–38]. However, in the present system, the TM contribution is thought to be negligible because the reaction (1) almost occurs from the singlet excited state as a monophotonic process. In addition, the polarization on e^-_{solv} was not observed although the e^-_{solv} lifetime was 0.61 μs in ethanol. The RTPM contribution might be possible because the lifetime of α-Toc• is so long that the interaction would occur between α-Toc• and some triplet state molecules, for example, when small amount of α-tocopherolquinone exists in the system. The *E*/*A* distorted polarization is usually due to the contribution of the ST_0 mixing (ST_0M) radical-pair mechanism (RPM) of CIDEP with the triplet precursor pair or the F-pair having the negative exchange integral ($J < 0$) [23,32–34,37,38]. The relatively large difference in *g*-value between e^-_{solv} ($g = 2.000$) and α-Toc• (2.0046) is expected to give an *E**/*A* (the low-field side is emissive and the high-field side absorptive with excess emission) polarization to α-Toc• through ST_0M-RPM [37,38]. In contrast, an *A**/*E* (the low-field side is absorptive and the high-field side emissive with excess absorption) polarization should be observed in the case of the singlet geminate-pair ST_0M-RPM in $J < 0$. The result might be explained with an overlap of the *A**/*E* polarization by the geminate-pair RPM and the *E**/*A* polarization by the F-pair RPM. On the other hand, it was reported that the geminate radical-pair of the phenoxyl radical (PhO•) and e^-_{solv} observed in the photoionization of phenol and *p*-cresol in aqueous solutions had the positive exchange integral ($J > 0$) [37,38]. In such situations, the geminate-pair ST_0M-RPM should give an *E**/*A* polarization to PhO• and e^-_{solv}. Therefore, the present TR-EPR spectra for α-Toc• might be explained by the singlet-precursor geminate-pair RPM ($J > 0$) polarization. Another explanation based on the radical-singlet-pair mechanism (RSPM) which can produce net and hyperfine-dependent polarization might also be possible. However, the RSPM contribution is thought to be minor because the lifetime of the singlet excited state in this system is very short. In this system, judging from very small signal intensity of the TR-EPR spectra, the spin polarization might come from minor processes.

4. Conclusion

In this study, we investigated the photochemical formation and decay processes of Toc• in the VE emulsion system by transient absorption spectroscopy, time-resolved EPR, and steady-state EPR. The photodecomposition of TocH is represented by reaction (1), that is, the electron-detachment of TocH in the excited state and the subsequent H^+ releasing, which produce Toc• and e^-_{solv}. The Toc• decay consists of three processes: (i) the rapid decay within 2 μs by the recombination between Toc• and e^-_{solv} (reaction (3)), (ii) the second-order decay observed in the range of 5–150 μs by the reaction between Toc• and $O_2^{\bullet -}$ (reaction (5)), and (iii) the slow decay in >10 ms by the bimolecular self-quenching of Toc• (reaction (4)). The Toc• decay in the VE emulsion was very different from that in the homogeneous solution. The Toc• yield in the VE emulsion was smaller than that in the homogeneous solutions. This is thought to come from the reduction of the electron-detachment efficiency of the excited TocH in E/W and the UV light-scattering of the emulsion. The contribution of reaction (3) in the VE emulsion was rather smaller than that in the homogeneous solutions. A rapid quenching process of e^-_{solv} in the VE emulsion other than reactions (2) and (3) reduced $O_2^{\bullet-}$ yield. The decay due to the bimolecular reaction of Toc• (reaction (4)) in the emulsion was much faster than that in the homogeneous solutions. This large acceleration should be caused by the oil-droplet formation. Lipophilic Toc• may be collected near the hydrophobic oil-droplet, and the reaction among Toc• occurs in the interface region around the oil-droplet. As a result, the reaction among Toc• is accelerated by reason of large local concentration of Toc•. In the emulsion, Toc• could not be detected by steady-state EPR, although it could in ethanol. The reason for this is the short lifetime (∼70 ms) and the small yield of Toc• in the emulsion. All these differences should be caused by the oil-droplet formation in the emulsion. From these results, we can expect that the photostability of the VE emulsion is superior to that of VE in the homogeneous solutions.

Acknowledgement

This work was supported by Grant-in-Aid for Scientific Research C (16550016 and 19550019) from the Japan Society for the Promotion of Science (JSPS).

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