JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Kinetic Study of Aroxyl Radical Scavenging and α-Tocopheroxyl Regeneration Rates of Pyrroloquinolinequinol ($PQQH_2$, a Reduced Form of Pyrroloquinolinequinone) in Dimethyl Sulfoxide Solution: Finding of Synergistic Effect on the Reaction Rate due to the Coexistence of α-Tocopherol and $PQQH_2$

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ABSTRACT: Measurements of aroxyl radical ($ArO^•$)-scavenging rate constants (k_s^{AOH}) of antioxidants (AOHs: pyrroloquinolinequinol ($PQQH_2$), α-tocopherol (α-TocH), ubiquinol-10 ($UQ_{10}H_2$), epicatechin, epigallocatechin, epigallocatechin gallate, and caffeic acid) were performed in dimethyl sulfoxide (DMSO) solution, using stopped-flow spectrophotometry. The k_s^{AOH} values were measured not only for each AOH but also for the mixtures of two AOHs ((i) α-TocH and $PQQH_2$ and (ii) α-TocH and $UQ_{10}H_2$). A notable synergistic effect that the k_s^{AOH} values increase 1.72, 2.42, and 2.50 times for α-TocH, $PQQH_2$, and $UQ_{10}H_2$, respectively, was observed for the solutions including two kinds of AOHs. Measurements of the regeneration rates of α-tocopheroxyl radical (α-$Toc^•$) to α-TocH by $PQQH_2$ and $UQ_{10}H_2$ were performed in DMSO, using double-mixing stopped-flow spectrophotometry. Second-order rate constants (k_r) obtained for $PQQH_2$ and $UQ_{10}H_2$ were 1.08×10^5 and 3.57×10^4 M^{-1} s^{-1}, respectively, indicating that the k_r value of $PQQH_2$ is 3.0 times larger than that of $UQ_{10}H_2$. It has been clarified that $PQQH_2$ and $UQ_{10}H_2$ having two HO groups within a molecule may rapidly regenerate two molecules of α-$Toc^•$ to α-TocH. The result indicates that the prooxidant effect of α-$Toc^•$ is suppressed by the coexistence of $PQQH_2$ or $UQ_{10}H_2$.

KEYWORDS: *free radicals, pyrroloquinolinequinone, pyrroloquinolinequinol, vitamin E, ubiquinol-10, antioxidant activity, reaction rate, stopped-flow spectrophotometry, coexistence of antioxidants, synergistic effect*

INTRODUCTION

Pyrroloquinolinequinone (PQQ) is a water-soluble quinone compound first identified as a a cofactor of alcohol- and glucose-dehydrogenases in bacteria.[1,2] PQQ has been receiving much attention in recent years, owing to its several interesting physiological functions.[3,4] PQQ is regarded to be a nutritionally important growth factor, because PQQ-deficient diets cause impaired growth, immunological defects, and decreased fertility in mice.[5] PQQ is related to mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1α expression.[6,7] Moreover, PQQ is related to regeneration of peripheral and central nerves. In *in vitro* experiments, PQQ enhances nerve growth factor, one of the neurotrophic factors responsible for the maintenance and development of peripheral nerves.[8] Regeneration of transected sciatic nerve in an *in vivo* rat model has been demonstrated.[9] It has been suggested that PQQ protects against secondary damage by attenuating inducible nitric oxide synthase (iNOS) expression following a primary physiological injury to spinal cord.[10] A small amount of PQQ has been found not only in microorganisms but also in human and rat organs or tissues, especially in the highest quantity in human milk.[11,12] An additional small amount of PQQ is also found in daily foods and beverages.[13,14]

Previous studies demonstrated that the reduced form of PQQ ($PQQH_2$ (pyrroloquinolinequinol), see Figure 1) exhibits antioxidative capacity in *in vitro* examinations.[15,16] We found that $PQQNa_2$ (disodium salt of PQQ) is easily reduced to $PQQH_2$, by reacting $PQQNa_2$ with glutathione and cysteine in buffer solution (pH 7.4) under nitrogen atmosphere.[17] This result suggests that PQQ exists as a reduced form throughout the cell and plays a role as an antioxidant. In experiments using cultured cells, it was reported that PQQ prevents oxidative stress-induced neuronal death.[18,19] Moreover, marked decreases in ischemia damage are found in *in vivo* models such as cardiovascular or cerebral ischemia models.[20,21] Furthermore, it was reported that PQQ prevents cognitive deficit caused by oxidative stress in rats.[22,23]

Lipid peroxyl radical ($LOO^•$) and singlet oxygen ($^1O_2$) are well-known as two representative reactive oxygen species generated in biological systems. In previous work, a kinetic study of the aroxyl radical ($ArO^•$)-scavenging activity of $PQQH_2$ and water-soluble antioxidants (AOHs) (such as vitamin C (Vit C), uric acid (UA), cysteine, and glutathione) was performed in 5.0 wt % Triton X-100 micellar solution (pH 7.4) using stopped-flow spectrophotometry (see reaction 1).[17] A stable aroxyl radical ($ArO^•$) (2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl) (see Figure 1) was used as a model of $LOO^•$ radical, as described in previous studies.[24−26]

Received: September 10, 2013
Revised: October 30, 2013
Accepted: October 31, 2013
Published: October 31, 2013

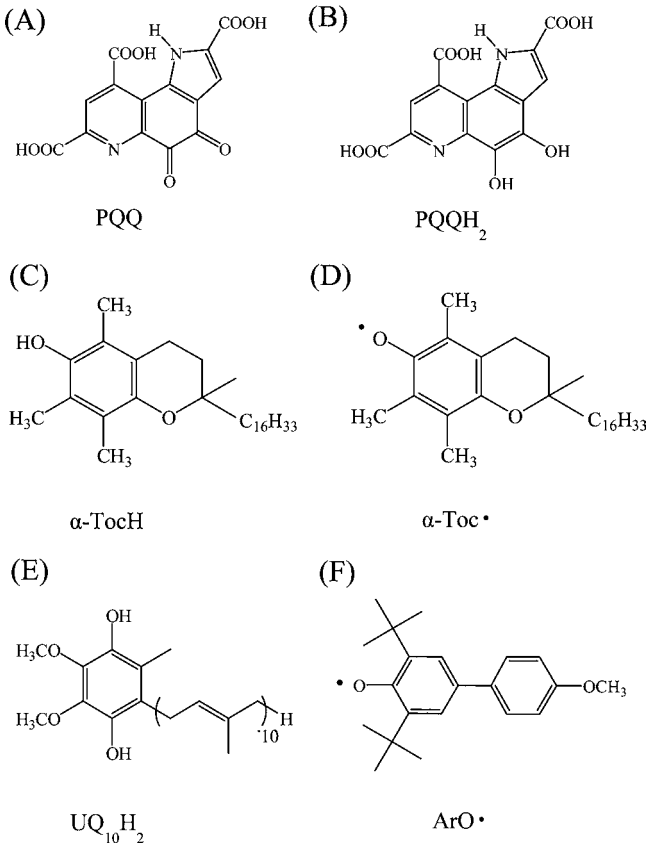

Figure 1. Molecular structures of pyrroloquinolinequinone (PQQ), pyrroloquinolinequinol ($PQQH_2$), α-tocopherol (α-TocH), α-tocopheroxyl radical (α-Toc•), ubiquinol-10 ($UQ_{10}H_2$), and aroxyl radical (ArO•).

The second-order rate constants (k_s) for the reaction of ArO• with $PQQH_2$ was found to be 7.4 times larger than that of Vit C, which is well-known as the most active water-soluble AOH.

$$ArO^{\bullet} + PQQH_2 \xrightarrow{k_s} ArOH + PQQH^{\bullet} \quad (1)$$

Furthermore, a kinetic study of the quenching reaction of $^1O_2$ with $PQQH_2$, $PQQNa_2$, and seven natural AOHs (Vit C, UA, epicatechin (EC), epigallocatechin (EGC), α-tocopherol (α-TocH), ubiquinol-10 ($UQ_{10}H_2$), and β-carotene (β-Car)) (reaction 2) has been performed in 5.0 wt % Triton X-100 micellar solution (pH 7.4), indicating that $PQQH_2$ shows high $^1O_2$-quenching activity.[27]

$$^1O_2 + PQQH_2 \xrightarrow{k_Q} {}^3O_2 + PQQH_2 \quad (2)$$

These results suggest that $PQQH_2$ may contribute to the protection of oxidative damage in biological systems by scavenging free radicals and quenching $^1O_2$.

In the present study, measurements of the second-order rate constant (k_s) were performed for reaction of ArO• radical with $PQQH_2$ and representative lipid- and water-soluble AOHs (such as α-TocH, $UQ_{10}H_2$, three catechins, and caffeic acid (CA); reaction 1) in dimethyl sulfoxide (DMSO) solution at 25 °C using stopped-flow spectrophotometry. Measurements of the k_s value were also performed for the solutions including two kinds of AOHs ((i) α-TocH and $PQQH_2$ and (ii) α-TocH and $UQ_{10}H_2$), in order to investigate the synergistic effect of AOHs on the ArO• radical-scavenging rate. Furthermore, measurements of the rate constant (k_r) for reactions of α-tocopheroxyl (α-Toc•) radical with $PQQH_2$ and $UQ_{10}H_2$ (reaction 3) were performed in DMSO solution using double-mixing stopped-flow spectrophotometry.

$$\alpha\text{-Toc}^{\bullet} + PQQH_2 \xrightarrow{k_r} ArOH + PQQH^{\bullet} \quad (3)$$

MATERIALS AND METHODS

Materials. A brown colored powder sample of $PQQH_2$ was supplied from Mitsubishi Gas Chemical Company, Inc. α-Tocopherol (α-TocH) and ubiquinone-10 (UQ_{10}) were kindly supplied from Eisai Co. Ltd. and Kaneka Co. Ltd., Japan, respectively. Epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) were kindly supplied from Mitsui Norin Co., Ltd., Japan. Caffeic acid (CA) was obtained from Nacalai Tesque, Japan. Ubiquinol-10 ($UQ_{10}H_2$) was prepared by the reduction of UQ_{10} with sodium hydrosulfite in *n*-hexane under a nitrogen atmosphere.[26] ArO• radical was prepared according to the method of Rieker and Scheffler.[28]

Methods. Measurement of the second-order rate constant (k_s) for the reaction of ArO• with AOH (reaction 1) was performed with a Unisoku single-mixing stopped-flow spectrophotometer (model RSP-1000) by mixing equal volumes of DMSO solutions of ArO• and AOH under nitrogen atmosphere.[17,24−26] The k_r values of $PQQH_2$ and $UQ_{10}H_2$ (reaction 3) were measured with a Unisoku double-mixing stopped-flow spectrophotometer (model RSP-1000-03F). The details of measurements were reported in previous studies.[29] All the measurements were performed at 25.0 ± 0.5 °C. Experimental errors in the rate constants (k_s and k_r) were estimated to be about 5% in DMSO solution. Ethanol had been generally used for measurements of the k_s and k_r values of AOHs in previous studies. However, DMSO was used in the present study, because the solubility of $PQQH_2$ is low in ethanol. $PQQH_2$ is stable in DMSO solution.

RESULTS

Measurements of the Aroxyl Radical Scavenging Rates (k_s (alone)) for $PQQH_2$ and Six Natural Antioxidants in DMSO Solution. Measurement of the rate constant (k_s) for reaction of ArO• radical with α-TocH was performed in DMSO solution (reaction 1). By reacting α-TocH with ArO• radical, the absorbance at 380 and 587 nm of the ArO• decreases, and the absorbance at 428 nm of α-Toc• radical increases, as shown in Figure 2A. The scavenging rate of ArO• was measured by following the decrease in absorbance at 380 or 587 nm of the ArO• radical, as shown in Figure 2B.[17,24−26] The pseudo-first-order rate constants (k_{obsd}) at 380 or 587 nm were linearly dependent on the concentration of α-TocH ([α-TocH]), and thus, the rate equation is expressed as

$$-d[ArO^{\bullet}]/dt = k_{obsd}[ArO^{\bullet}] = k_s[\alpha\text{-TocH}][ArO^{\bullet}] \quad (4)$$

where k_s is the second-order rate constant for oxidation of α-TocH by ArO• radical. The rate constant (k_s) was obtained by plotting k_{obsd} against [α-TocH], as shown in Figure 2C. The $k_s^{\alpha\text{-TocH}}$ (alone) value obtained is 7.02×10^2 M^{-1} s^{-1} (see Table 1), where "$k_s^{\alpha\text{-TocH}}$ (alone)" means the ArO•-radical scavenging rate constants obtained in solution including only one component of AOH.

As described above, by reacting α-TocH with ArO• in DMSO, α-Toc• is produced rapidly. α-Toc• is unstable at 25.0 °C; its absorption peak decreases gradually after passing though the maximum and disappears by a bimolecular reaction (reaction 5; see Figure 2D).[30]

$$\alpha\text{-Toc}^{\bullet} + \alpha\text{-Toc}^{\bullet} \xrightarrow{2k_d} \text{nonradical products (NRP)} \quad (5)$$

As shown in Figure 2D, the maximum absorbance of α-Toc• observed at t_{max} increases with increasing [α-TocH] and

dx.doi.org/10.1021/jf4040496 | J. Agric. Food Chem. 2013, 61, 11048−11060

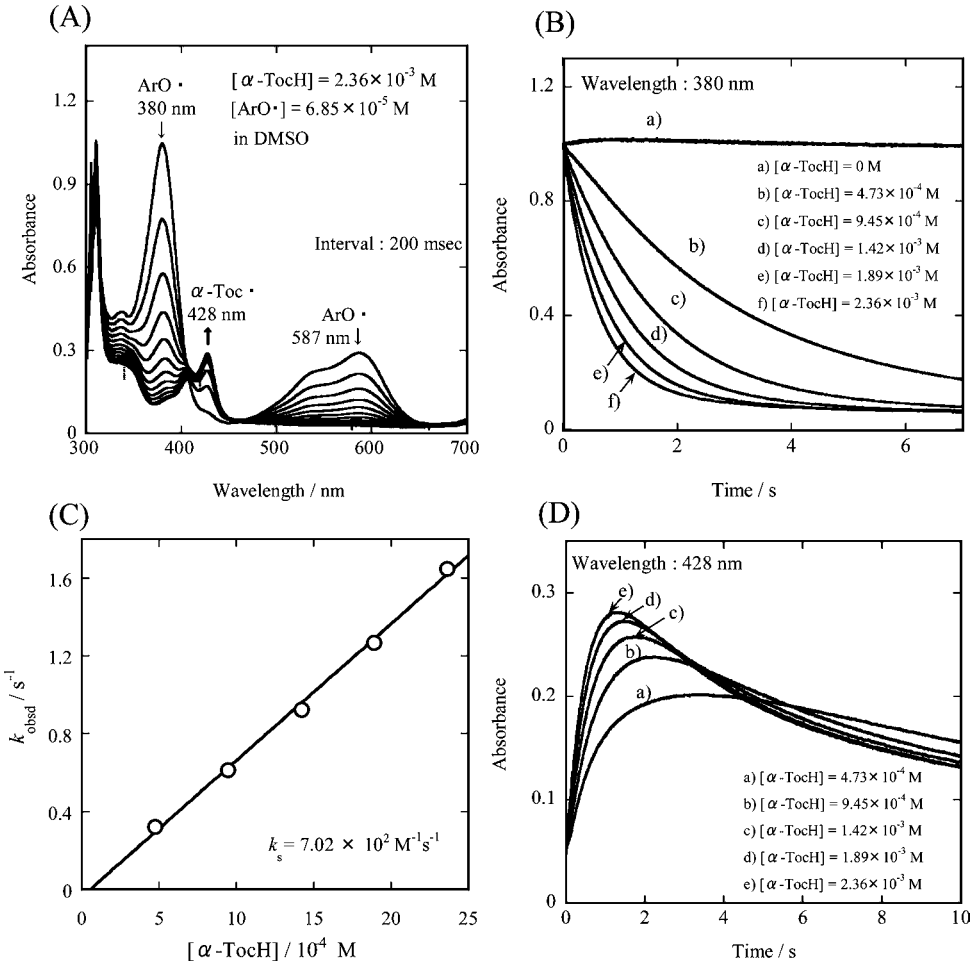

Figure 2. (A) Change in electronic absorption spectra of ArO• and α-Toc• radicals during reaction of ArO• with α-TocH in DMSO solution at 25.0 °C. Initial concentration is [ArO•] = 6.85 × 10^{-5} M and [α-TocH] = 2.36 × 10^{-3} M. Spectra were recorded at 200 ms intervals. Arrow indicates a decrease (ArO•) and an increase (α-Toc•) in absorbance with time. Time dependences of the absorbance of (B) ArO• radical (at 380 nm) and (D) α-Toc• radical (at 428 nm) in solutions including six and five different concentrations of α-TocH at 25.0 °C, respectively. (C) Pseudo-first-order rate constant (k_{obsd}) versus [α-TocH] plot.

Table 1. Second-Order Rate Constants (k_s^{AOH} (alone)) for Reaction 1 of ArO• Radical with Seven Kinds of Antioxidants (AOHs), Relative Rate Constants (k_s^{AOH} (alone)/$k_s^{\alpha\text{-TocH}}$ (alone)), and Second-Order Rate Constants (k_r^{AOH}) for Reaction 3 of α-Toc• Radical with Ubiquinol-10 and $PQQH_2$ in DMSO Solution at 25.0 °C

antioxidant	k_s^{AOH} (alone),[a] M^{-1} s^{-1}	avg k_s^{AOH} (alone), M^{-1} s^{-1}[b]	k_s^{AOH} (alone)/$k_s^{\alpha\text{-TocH}}$ (alone)	k_r^{AOH},[a] M^{-1} s^{-1}
α-TocH	(7.31 ± 0.21) × 10^2	7.34 × 10^2	1.00	
	(7.02 ± 0.22) × 10^2			
	(7.47 ± 0.08) × 10^2			
	(7.55 ± 0.08) × 10^2			
	(7.36 ± 0.25) × 10^2			
$UQ_{10}H_2$	(1.16 ± 0.01) × 10^3	1.23 × 10^3	1.68	(3.57 ± 0.13) × 10^4
	(1.27 ± 0.02) × 10^3			
	(1.25 ± 0.02) × 10^3			
$PQQH_2$	(2.63 ± 0.04) × 10^2	2.52 × 10^2	0.343	(1.08 ± 0.02) × 10^5
	(2.54 ± 0.03) × 10^2			
	(2.43 ± 0.04) × 10^2			
	(2.48 ± 0.06) × 10^2			
EGCG	(1.58 ± 0.07) × 10^2	1.55 × 10^2	0.211	
	(1.52 ± 0.08) × 10^2			
EGC	(1.22 ± 0.03) × 10^2		0.166	
EC	(1.86 ± 0.06) × 10		0.0253	
CA	(1.09 ± 0.04) × 10		0.0149	

[a]Given as value ± standard deviation. [b]Avg denotes the average value.

dx.doi.org/10.1021/jf4040496 | J. Agric. Food Chem. 2013, 61, 11048−11060

approaches a constant value, because at high [α-TocH] α-Toc$^\bullet$ appears rapidly and the decay of α-Toc$^\bullet$ is very small and negligible. Therefore, we can estimate the ε value of α-Toc$^\bullet$ radical at λ_{max} = 428 nm by assuming that [α-Toc$^\bullet$] at t_{max} equals [ArO$^\bullet$] at t = 0 s and using Lambert–Beer's equation (absorbance (of α-Toc$^\bullet$ at t_{max}) = $\varepsilon \times$ [ArO$^\bullet$]$_{t=0}$), as reported in a previous study.[30] The ε value of α-Toc$^\bullet$ radical obtained was 3930 M^{-1} cm^{-1} in DMSO solution.

Similarly, by reacting $UQ_{10}H_2$ with ArO$^\bullet$ radical, the absorbance at 380 nm of ArO$^\bullet$ decreases rapidly, as shown in Figure 3A. The rate constant ($k_s^{UQ_{10}H_2}$ (alone)) was obtained by plotting k_{obsd} against [$UQ_{10}H_2$] (see Figure 3B). As shown in Figure 3A, we could not observe the absorption spectrum of $UQ_{10}H^\bullet$ radical because of its instability.[31] By reaction of $PQQH_2$ with ArO$^\bullet$, the absorbance at 587 nm of the ArO$^\bullet$ decreases rapidly, as shown in Figure 4A. Measurement of the decay curve was performed at 610 nm, because strong absorption of $PQQH_2$ overlaps that of ArO$^\bullet$ at the wavelength region of 380–580 nm (see Figure 4A). The baseline corrections were performed by using the value of ε = 31.7 M^{-1} cm^{-1} at 610 nm of $PQQH_2$ in DMSO, as shown in Figure 4B. The rate constant ($k_s^{PQQH_2}$ (alone)) was obtained by plotting k_{obsd} against [$PQQH_2$] (see Figure 4C).

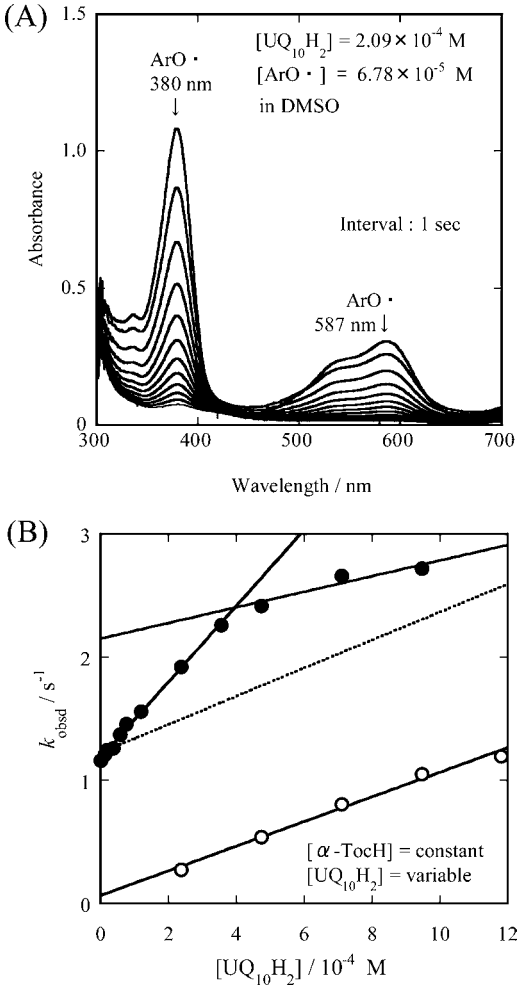

Figure 3. (A) Change in electronic absorption spectrum of ArO$^\bullet$ radical during reaction of ArO$^\bullet$ with $UQ_{10}H_2$ in DMSO at 25.0 °C. Initial concentration is [ArO$^\bullet$] = 6.78 × 10^{-5} M and [$UQ_{10}H_2$] = 2.09 × 10^{-4} M. Absorption of $UQ_{10}H^\bullet$ was not observed. (B) Plots of k_{obsd} versus [$UQ_{10}H_2$] for reactions of ArO$^\bullet$ radical with (i) $UQ_{10}H_2$ only (○) and (ii) mixture of α-TocH and $UQ_{10}H_2$ (●) (see Table 2B). Dotted line shows the plots for which any synergistic effect is absent between α-TocH and $UQ_{10}H_2$.

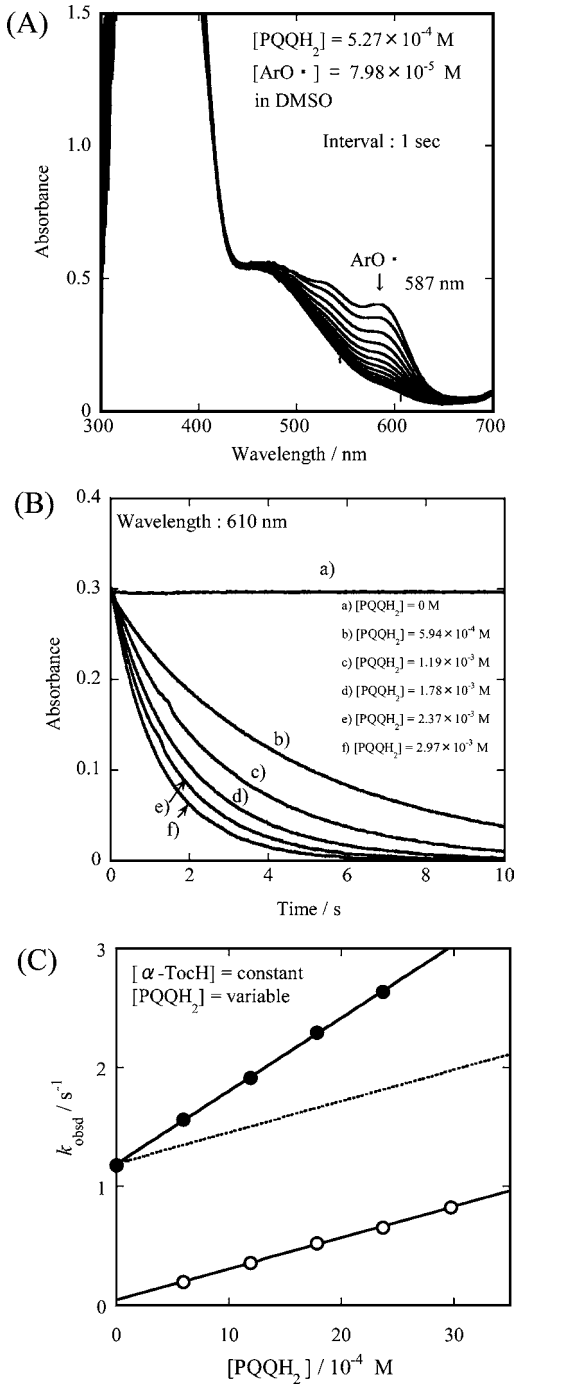

Figure 4. (A) Change in electronic absorption spectrum of ArO$^\bullet$ radical during reaction of ArO$^\bullet$ with $PQQH_2$ in DMSO at 25.0 °C. [ArO$^\bullet$] = 7.98 × 10^{-5} M and [$PQQH_2$] = 5.27 × 10^{-4} M. (B) Time dependences of the absorbance of ArO$^\bullet$ radical at 610 nm in DMSO solutions including six different concentrations of $PQQH_2$, where the correction of baseline due to $PQQH_2$ was performed (see text). (C) Plots of k_{obsd} versus [$PQQH_2$] for reactions of ArO$^\bullet$ radical with (i) $PQQH_2$ only (○) and (ii) mixture of α-TocH and $PQQH_2$ (●) (see Table 2B). Dotted line shows the plots for which any synergistic effect is absent between α-TocH and $PQQH_2$.

dx.doi.org/10.1021/jf4040496 | J. Agric. Food Chem. 2013, 61, 11048–11060

Measurements of the k_s^{AOH} (alone) values were repeated 3−5 times for every α-TocH, $UQ_{10}H_2$, and $PQQH_2$ to obtain reliable k_s^{AOH} (alone) values. Each k_s^{AOH} (alone) value and average k_s^{AOH} (alone) values (showing experimental errors) obtained are summarized in Table 1. The k_s^{AOH} (alone) values of $UQ_{10}H_2$ and $PQQH_2$ are 1.68 and 0.343 times as large as that of α-TocH, respectively.

Furthermore, the k_s^{AOH} (alone) values were measured for four kinds of well-known phenolic AOHs (EC, EGC, EGCG, and CA) to compare the k_s^{AOH} (alone) values with that of $PQQH_2$. The results obtained are listed in Table 1, together with those of α-TocH, $UQ_{10}H_2$, and $PQQH_2$. The k_s^{AOH} (alone) values obtained decreased in the order of eq 6.

$$UQ_{10}H_2 > \alpha\text{-TocH} > PQQH_2 > EGCG > EGC > EC > CA \quad (6)$$

The k_s^{AOH} (alone) value of $PQQH_2$ was greater than those of hydrophilic AOHs (EGCG, EGC, EC, and CA) in DMSO solution. However, it was less than those of lipophilic AOHs ($UQ_{10}H_2$ and α-TocH).

Measurements of the Regeneration Rates (k_r) of α-Tocopherol by $PQQH_2$ and Ubiquinol-10 in DMSO Solution. As described in the Methods section, measurement of the k_r value for reaction of α-Toc$^\bullet$ radical with $UQ_{10}H_2$ (reaction 3) was performed in DMSO solution, using a double-mixing stopped-flow spectrophotometer.[29] α-Toc$^\bullet$ radical was prepared by the first-mixing of equal volumes of α-TocH (cell A) and ArO$^\bullet$ (cell B) solutions (reaction 1), and after 2 s the second-mixing of equal volumes of α-Toc$^\bullet$ solution and $UQ_{10}H_2$ solution (cell C) (reaction 3) was made. The typical concentrations in cells A and B are 7.24×10^{-3} and 5.14×10^{-4} M, respectively. The decay curves of the absorbance of α-Toc$^\bullet$ at 428 nm in DMSO are shown in Figure 5A, indicating that the decay rates increase with increasing $[UQ_{10}H_2]$.

The pseudo first-order rate constants (k_{obsd}) observed at 428 nm were linearly dependent on $[UQ_{10}H_2]$, and thus, the rate equation is expressed as follows:

$$-d[\alpha\text{-Toc}^\bullet]/dt = k_{obsd}[\alpha\text{-Toc}^\bullet] = k_r[UQ_{10}H_2][\alpha\text{-Toc}^\bullet] \quad (7)$$

The k_r value was obtained by plotting k_{obsd} against $[UQ_{10}H_2]$, as shown in Figure 5B.

Similar measurements were performed for the reaction of α-Toc$^\bullet$ with $PQQH_2$ in DMSO solution. α-Toc$^\bullet$ radical was prepared by the first-mixing of equal volumes of α-TocH and ArO$^\bullet$ solutions, and after 2 s the second-mixing of equal volumes of α-Toc$^\bullet$ solution and $PQQH_2$ solution (reaction 3) was performed (see Figure 6A). The decay curves of the absorbance of α-Toc$^\bullet$ at 428 nm in DMSO are shown in Figure 6B. The baseline corrections were performed by using the value of $\varepsilon = 1120\ M^{-1}\ cm^{-1}$ at 428 nm of $PQQH_2$ in DMSO. Decay curves obtained by baseline correction are shown in Figure 6C, indicating that the decay rates increase with increasing concentrations of $PQQH_2$.

The k_{obsd} versus $[PQQH_2]$ plot is also shown in Figure 5B. k_r values obtained for $UQ_{10}H_2$ and $PQQH_2$ are 3.57×10^4 and $1.08 \times 10^5\ M^{-1}\ s^{-1}$, respectively, as listed in Table 1. The rate constant (k_r) of $PQQH_2$ is 3.0 times larger than that of $UQ_{10}H_2$. k_r values obtained are very fast and about 2−3 orders of magnitude larger than those for $k_s^{\alpha\text{-TocH}}$ (alone) (avg $7.34 \times 10^2\ M^{-1}\ s^{-1}$), $k_s^{UQ_{10}H_2}$ (alone) (avg $1.23 \times 10^3\ M^{-1}\ s^{-1}$), and $k_s^{PQQH_2}$ (alone) (avg $2.52 \times 10^2\ M^{-1}\ s^{-1}$).

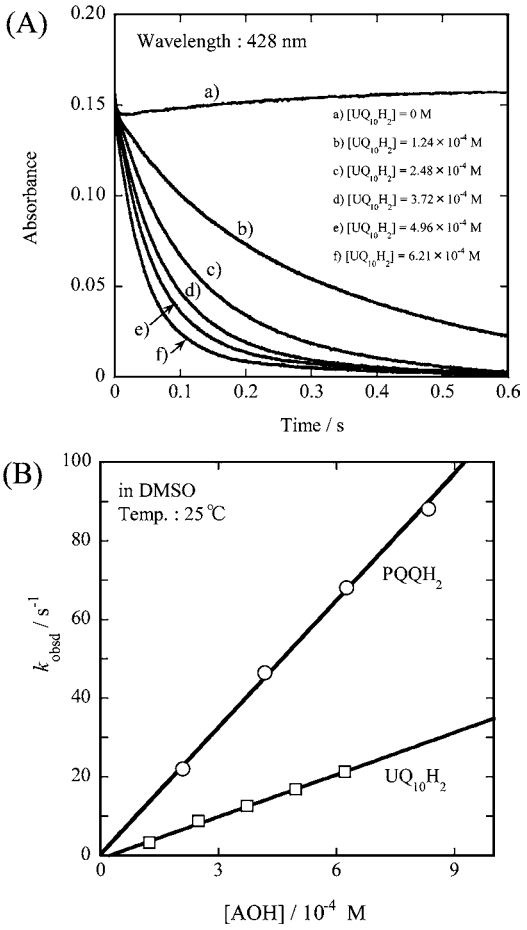

Figure 5. (A) Time dependences of the absorbance of α-Toc$^\bullet$ radical at 428 nm in DMSO including six different concentrations of $UQ_{10}H_2$ at 25.0 °C. (B) k_{obsd} versus [AOH] (AOH = $UQ_{10}H_2$ or $PQQH_2$) plot.

Measurements of the Aroxyl Radical-Scavenging Rates (k_s) for Mixtures of α-Tocopherol and Ubiquinol-10 in DMSO. Measurements of the $k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$) and $k_s^{UQ_{10}H_2}$ (+α-TocH) values were performed for the solution including α-TocH and $UQ_{10}H_2$. the terms "$k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$) and $k_s^{UQ_{10}H_2}$ (+α-TocH)" indicate the rate constants obtained in the solution including two components of AOHs. First, the $k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$) value was measured by keeping $[UQ_{10}H_2]$ constant (4.96×10^{-4} M) and varying [α-TocH] (0 to 1.89×10^{-3} M). By mixing the solution of ArO$^\bullet$ radical with the solution including α-TocH and $UQ_{10}H_2$, absorption of the ArO$^\bullet$ at 380 and 587 nm decreases rapidly, as shown in Figure 7A. Absorption of α-Toc$^\bullet$ at 428 nm was not observed at low [α-TocH] (0 to 9.45×10^{-4} M), but weak absorption appeared at high [α-TocH] ($(1.42-1.89) \times 10^{-3}$ M); see Figure 7B). By analysis of the decay curves of ArO$^\bullet$ at 380 nm, k_{obsd} values were determined. Figure 7C shows the k_{obsd} versus [α-TocH] plot.

If α-TocH and $UQ_{10}H_2$ coexist in solution, reactions 8 and 9 will occur competively in solution, because the difference in the rate constants is less than two times, as listed in Table 1.

$$ArO^\bullet + \alpha\text{-TocH} + UQ_{10}H_2 \xrightarrow{k_s^{\alpha\text{-TocH}}} ArOH + \alpha\text{-Toc}^\bullet + UQ_{10}H_2 \quad (8)$$

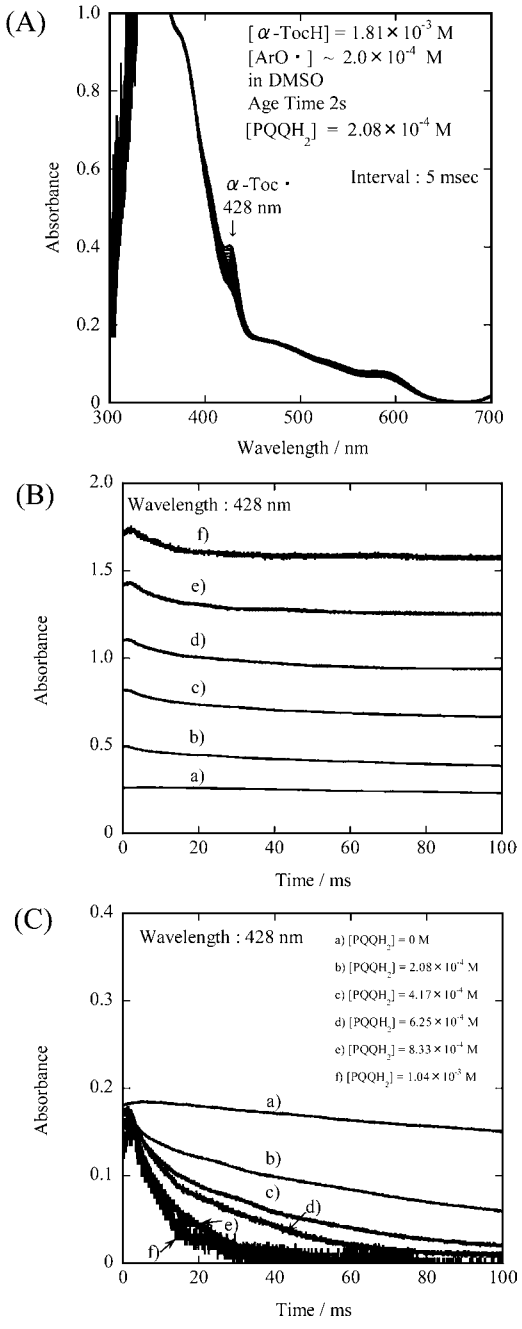

Figure 6. (A) Change in absorption spectrum (at 5 ms intervals) during reaction 3 of $PQQH_2$ and α-Toc$^\bullet$ in DMSO at 25.0 °C. Initial concentration of $[PQQH_2]$ in cell C was 2.08 × 10^{-4} M. Arrow indicates a decrease in absorbance of α-Toc$^\bullet$. (B) Absorbance decay of α-Toc$^\bullet$ at 428 nm during reaction 3 in DMSO at 25.0 °C. (C) Baseline corrections for the absorption of $PQQH_2$ at 428 nm were performed.

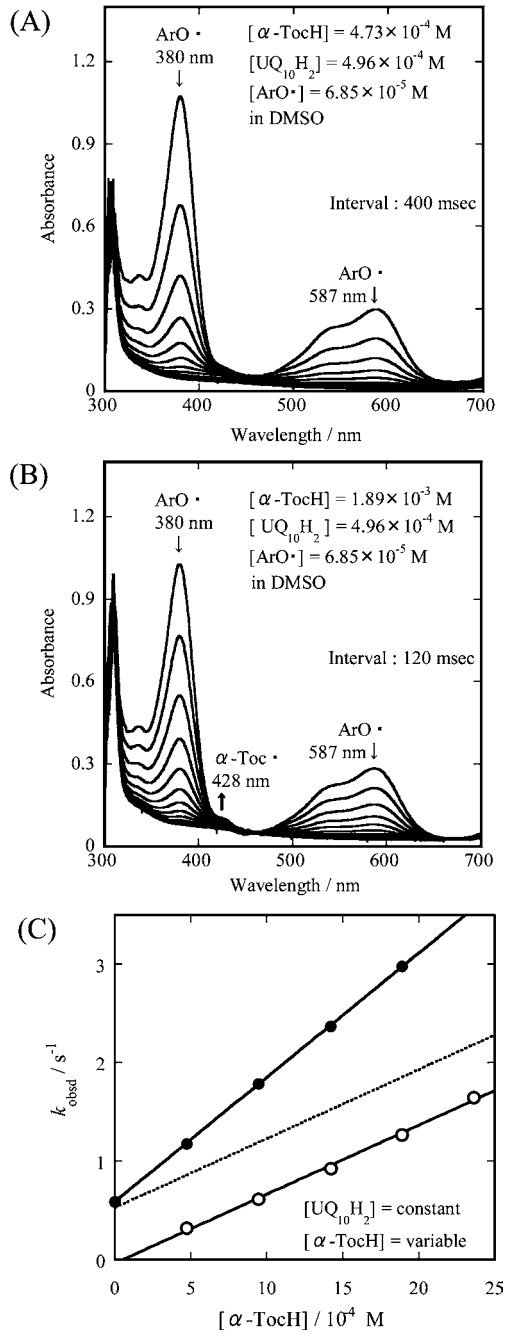

Figure 7. Change in electronic absorption spectrum of ArO$^\bullet$ radical during reaction of ArO$^\bullet$ with a mixture of α-TocH and $UQ_{10}H_2$ in DMSO at 25.0 °C. [ArO$^\bullet$] = 6.85 × 10^{-5} and $[UQ_{10}H_2]$ = 4.96 × 10^{-4} M. (A) [α-TocH] = 4.73 × 10^{-4} M and (B) [α-TocH] = 1.89 × 10^{-3} M. (C) Plots of k_{obsd} versus [α-TocH] for reactions of ArO$^\bullet$ radical with (i) α-TocH only (○) and (ii) mixture of α-TocH and $UQ_{10}H_2$ (●) (see Table 2A). Dotted line shows the plots for which any synergistic effect is absent between α-TocH and $UQ_{10}H_2$.

$$\text{ArO}\bullet + \alpha\text{-TocH} + UQ_{10}H_2 \xrightarrow{k_s^{UQ_{10}H_2}} \text{ArOH} + \alpha\text{-TocH} + UQ_{10}H^\bullet \quad (9)$$

In such a case, we can expect that the k_{obsd} value depends on eq 10, if the interaction between α-TocH and $UQ_{10}H_2$ is negligible.

$$k_{obsd} = k_s^{\alpha\text{-TocH}}(\text{alone})[\alpha\text{-TocH}] + k_s^{UQ_{10}H_2}(\text{alone})[UQ_{10}H_2] \quad (10)$$

By substitution of $k_s^{\alpha\text{-TocH}}$ (alone) and $k_s^{UQ_{10}H_2}$ (alone) values and the value of $[UQ_{10}H_2]$ (4.96 × 10^{-4} M) used for measurement into eq 10, k_{obsd} was plotted against [α-TocH] (see a dotted line in Figure 7C). The result of the k_{obsd} versus [α-TocH] plot (open circle) obtained for the solution including only α-TocH is also shown in Figure 7C. As described above, measurement was performed, by keeping $UQ_{10}H_2$ at a constant concentration and varying [α-TocH]. Consequently, the $k_s^{\alpha\text{-TocH}}$

dx.doi.org/10.1021/jf4040496 | J. Agric. Food Chem. 2013, 61, 11048−11060

Table 2. Second-Order Rate Constants (k_s^{AOH} (+2nd AOH)) Obtained for Mixtures of Two Kinds of Antioxidants ((i) α-TocH and $UQ_{10}H_2$ and (ii) α-TocH and $PQQH_2$) and Ratios (k_s^{AOH} (+2nd AOH)/k_s^{AOH} (alone))

(A) Measurements Were Performed by Keeping [AOH] Constant and Varying [α-TocH]				
antioxidant (AOH)	[AOH], M	[α-TocH], M	$k_s^{\alpha\text{-TocH}}$ (+AOH),[a] M^{-1} s^{-1}	$k_s^{\alpha\text{-TocH}}$ (+AOH)/$k_s^{\alpha\text{-TocH}}$ (alone)
$UQ_{10}H_2$	4.96×10^{-4}	$(0-1.89) \times 10^{-4}$	$(1.26 \pm 0.01) \times 10^3$	1.72
$PQQH_2$	3.71×10^{-3}	$(0-1.84) \times 10^{-3}$	$(8.26 \pm 0.40) \times 10^2$	1.13
(B) Measurements Were Performed by Keeping [α-TocH] Constant and Varying [AOH]				
antioxidant (AOH)	[α-TocH], M	[AOH], M	k_s^{AOH} (+α-TocH),[a] M^{-1} s^{-1}	k_s^{AOH} (+α-TocH)/k_s^{AOH} (alone)
$UQ_{10}H_2$	1.67×10^{-3}	$(0-3.55) \times 10^{-4}$	$(3.08 \pm 0.08) \times 10^3$	2.50
		$(4.73-9.47) \times 10^{-4}$	$(6.33 \pm 2.19) \times 10^2$	0.515
$PQQH_2$	1.63×10^{-3}	$(0-3.06) \times 10^{-3}$	$(6.10 \pm 0.13) \times 10^2$	2.42

[a]Given as value ± standard deviation (sd).

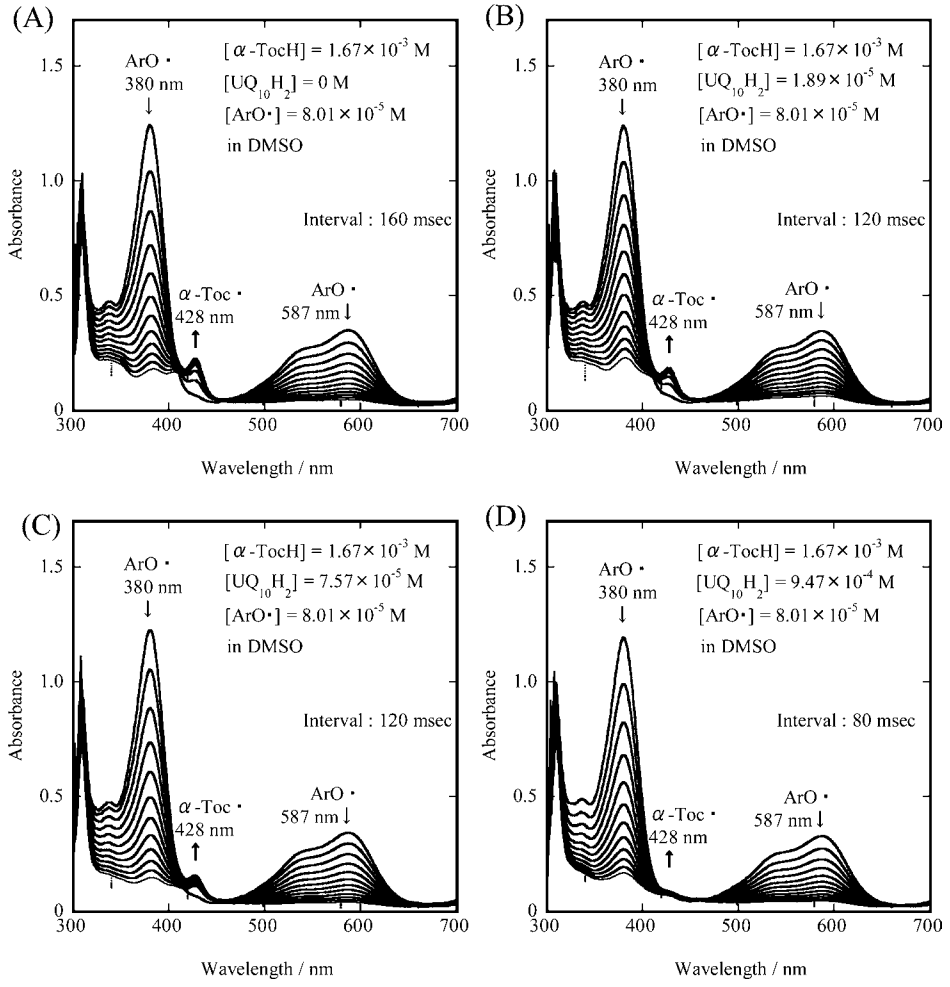

Figure 8. Change in electronic absorption spectra of ArO• and α-Toc• radicals during reaction of ArO• with mixture of α-TocH and $UQ_{10}H_2$ in DMSO at 25.0 °C. [ArO•] = 8.01×10^{-5} and [α-TocH] = 1.67×10^{-3} M. Spectra were recorded at (A)160 ms, (B) 120 ms, (C) 120 ms, and (D) 80 ms intervals. Absorption of α-Toc• decreased with increasing concentrations of $UQ_{10}H_2$. (A) [$UQ_{10}H_2$] = 0 M, (B) 1.89×10^{-5} M, (C) 7.57×10^{-5} M, and (D) 9.47×10^{-4} M.

(+$UQ_{10}H_2$) value was determined from the gradient of the k_{obsd} versus [α-TocH] plot (closed circle) in Figure 7C, using eq 11.

$$k_{obsd} = k_s^{\alpha\text{-TocH}}(+UQ_{10}H_2)[\alpha\text{-TocH}] + k_s^{UQ_{10}H_2}(\text{alone})[UQ_{10}H_2] \quad (11)$$

As expected from the gradient in Figure 7C, the $k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$) value (1.26×10^3 M^{-1} s^{-1}) is 1.72 times larger than $k_s^{\alpha\text{-TocH}}$ (alone) (7.34×10^2 M^{-1} s^{-1}) obtained for the solution including only α-TocH. A notable effect due to the coexistence of α-TocH and $UQ_{10}H_2$ in solution was observed for the rate constant ($k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$)).

As a notable effect of the coexistence of α-TocH and $UQ_{10}H_2$ was observed for the rate constants ($k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$)), similar measurements were performed for the solutions including α-TocH and $UQ_{10}H_2$, by keeping [α-TocH] constant and varying [$UQ_{10}H_2$] (see Table 2B). As shown in Figure 8A–D, the absorption of α-Toc• at 428 nm decreased with increasing the concentration of $UQ_{10}H_2$, suggesting that the α-Toc• produced is regenerated quickly to α-TocH by the

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reaction with $UQ_{10}H_2$ (reaction 3), because the regeneration rate constant (k_r = 3.57 × 10^4 M^{-1} s^{-1}) is very fast compared with the $k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$) value (1.26 × 10^3 M^{-1} s^{-1}).

As shown in Figure 3B, the k_{obsd} versus [$UQ_{10}H_2$] plot (closed circle) consists of two lines having different gradients. The analysis of the rate constant ($k_s^{UQ_{10}H_2}$ (+α-TocH)) was performed tentatively by using eq 12 similar to eq 11.

$$k_{obsd} = k_s^{\alpha\text{-TocH}}(\text{alone})[\alpha\text{-TocH}] + k_s^{UQ_{10}H_2}(+\alpha\text{-TocH})[UQ_{10}H_2] \quad (12)$$

The $k_s^{UQ_{10}H_2}$ (+α-TocH) values obtained at low and high [$UQ_{10}H_2$] ((0−3.55) × 10^{-4} and (4.73−9.47) × 10^{-4} M) are 3.08 × 10^3 and 6.33 × 10^2 M^{-1} s^{-1}, respectively (see Table 2B). The $k_s^{UQ_{10}H_2}$ (+α-TocH) values of the former and the latter are 2.50 and 0.515 times larger and smaller than the $k_s^{UQ_{10}H_2}$ (alone) value, respectively.

Measurements of the Aroxyl Radical-Scavenging Rates (k_s) for Mixtures of α-Tocopherol and $PQQH_2$ in DMSO. Similar measurements (see Table 2A) were performed for the solution including α-TocH and $PQQH_2$ by keeping [$PQQH_2$] constant and varying [α-TocH]. The scavenging rate (k_{obsd}) of $ArO^•$ was measured by following the decrease in absorbance at 587 nm of the $ArO^•$ radical (see Figure 9A).[17] The k_{obsd} versus [α-TocH] plot is shown in Figure 9B. As performed for mixture of α-TocH and $UQ_{10}H_2$ (see eq 11), the $k_s^{\alpha\text{-TocH}}$ (+$PQQH_2$) values were determined. The $k_s^{\alpha\text{-TocH}}$ (+$PQQH_2$) values (8.26 × 10^2 M^{-1} s^{-1}) obtained under the coexistence of $PQQH_2$ are 1.13 times larger than the $k_s^{\alpha\text{-TocH}}$ (alone) value (avg 7.34 × 10^2 M^{-1} s^{-1}).

The $k_s^{PQQH_2}$ (+α-TocH) value was measured by keeping [α-TocH] constant (1.63 × 10^{-3} M) and varying [$PQQH_2$] ((0−3.06) × 10^{-3} M) (see Table 2B). As shown in Figure 10A−D, absorption of α-$Toc^•$ at 428 nm decreased with increasing concentration of $PQQH_2$, suggesting that the α-$Toc^•$ produced is regenerated quickly to α-TocH by reaction with $PQQH_2$ (reaction 3), because the regeneration rate constant (k_r) (1.08 × 10^5 M^{-1} s^{-1}) is very fast compared with the $k_s^{\alpha\text{-TocH}}$ (+$PQQH_2$) value (8.26 × 10^2 M^{-1} s^{-1}).

Figure 4C shows the k_{obsd} versus [$PQQH_2$] plot. From the gradient, the $k_s^{PQQH_2}$ (+α-TocH) value (6.10 × 10^2 M^{-1} s^{-1}) was obtained. The value is 2.42 times larger than $k_s^{PQQH_2}$ (alone) (2.52 × 10^2 M^{-1} s^{-1}). Large synergistic effect was observed under the coexistence of α-TocH and $PQQH_2$.

Decrease in UV−Vis Absorption of α-Tocopheroxyl Radical under the Coexistence of α-Tocopherol and $PQQH_2$ (or Ubiquinol-10). Upon reaction of $ArO^•$ radical with α-TocH, absorption of α-$Toc^•$ radical appears rapidly and decreases gradually, as shown in Figure 2D. The concentration of α-$Toc^•$ radical ([α-$Toc^•$]) produced by reaction with $ArO^•$ ([$ArO^•$] = 6.85 × 10^{-5} M) is similar to that of $ArO^•$, if a high concentration of α-TocH was used for the reaction, as described in a previous section and reported in a previous study.[30]

On the other hand, if $UQ_{10}H_2$ coexists in the above solution, absorption of α-$Toc^•$ radical at 428 nm decreases greatly with increasing [$UQ_{10}H_2$] and disappears at higher [$UQ_{10}H_2$], as shown in Figure 8. The time dependence of [α-$Toc^•$] observed at λ_{max} (428 nm) is shown in Figure 11A, where [α-$Toc^•$] was calculated from the absorbance of α-$Toc^•$, using the relation (absorbance = εcl, ε = 3930 M^{-1} cm^{-1}).[30] Figure 11B shows the [α-$Toc^•$] (at 1.5 s in Figure 11A) versus [$UQ_{10}H_2$] plot, indicating that the [α-$Toc^•$] at 1.5 s decreases rapidly with

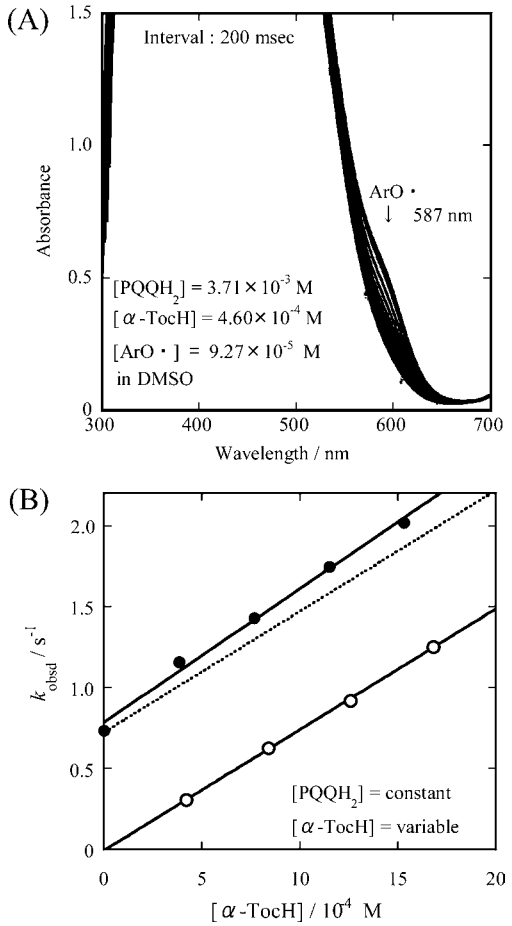

Figure 9. (A) Change in electronic absorption spectrum of $ArO^•$ radical during reaction of $ArO^•$ with a mixture of α-TocH and $PQQH_2$ in DMSO at 25.0 °C. [$ArO^•$] = 9.27 × 10^{-5}, [α-TocH] = 4.60 × 10^{-4}, and [$PQQH_2$] = 3.71 × 10^{-3} M. (B) Plots of k_{obsd} versus [α-TocH] for reaction of $ArO^•$ radical with (i) α-TocH only (○) and (ii) mixture of α-TocH and $PQQH_2$ (●) (see Table 2A). Dotted line shows the plots for which any synergistic effect is absent between α-TocH and $PQQH_2$.

increasing [$UQ_{10}H_2$]. As shown in Figure 11A, if [$UQ_{10}H_2$] ≤ 1.89 × 10^{-5} M (plot c), α-$Toc^•$ does not disappear at 10 s and remains in solution for a few minutes. However, if [$UQ_{10}H_2$] = 3.79 × 10^{-5} M (plot d), absorption of α-$Toc^•$ disappears at ∼7 s. The result suggests that one molecule of $UQ_{10}H_2$ quickly regenerates two molecules of α-$Toc^•$ to two molecules of α-TocH, because the initial [α-$Toc^•$] (= [$ArO^•$]) is 8.01 × 10^{-5} M (see Figures 8 and 11). Two HO groups in $UQ_{10}H_2$ molecule will contribute to the regeneration reaction of α-$Toc^•$ radical. With increasing [$UQ_{10}H_2$] ((d) 3.79 × 10^{-5}, (e) 5.68 × 10^{-5}, (f) 7.57 × 10^{-5}, (g) 1.18 × 10^{-4} M), the time at which absorption of α-$Toc^•$ disappears decreases in the order of ∼7, 5, 4, and 3.5 s, respectively.

Similar measurements were performed for solutions including α-TocH and $PQQH_2$. The time dependence of [α-$Toc^•$] observed at λ_{max} (428 nm) is shown in Figure 12A. Because the absorption of $PQQH_2$ overlaps that of α-$Toc^•$, baseline correction was performed (see Figure 12B). As shown in Figure 12B, [α-$Toc^•$] decreases abruptly with increasing [$PQQH_2$]. The [α-$Toc^•$] (at 1.5 s in Figure 12B) versus [$PQQH_2$] plot is shown in Figure 12C, indicating that the [α-$Toc^•$] at 1.5 s decreases rapidly with increasing [$PQQH_2$]. As

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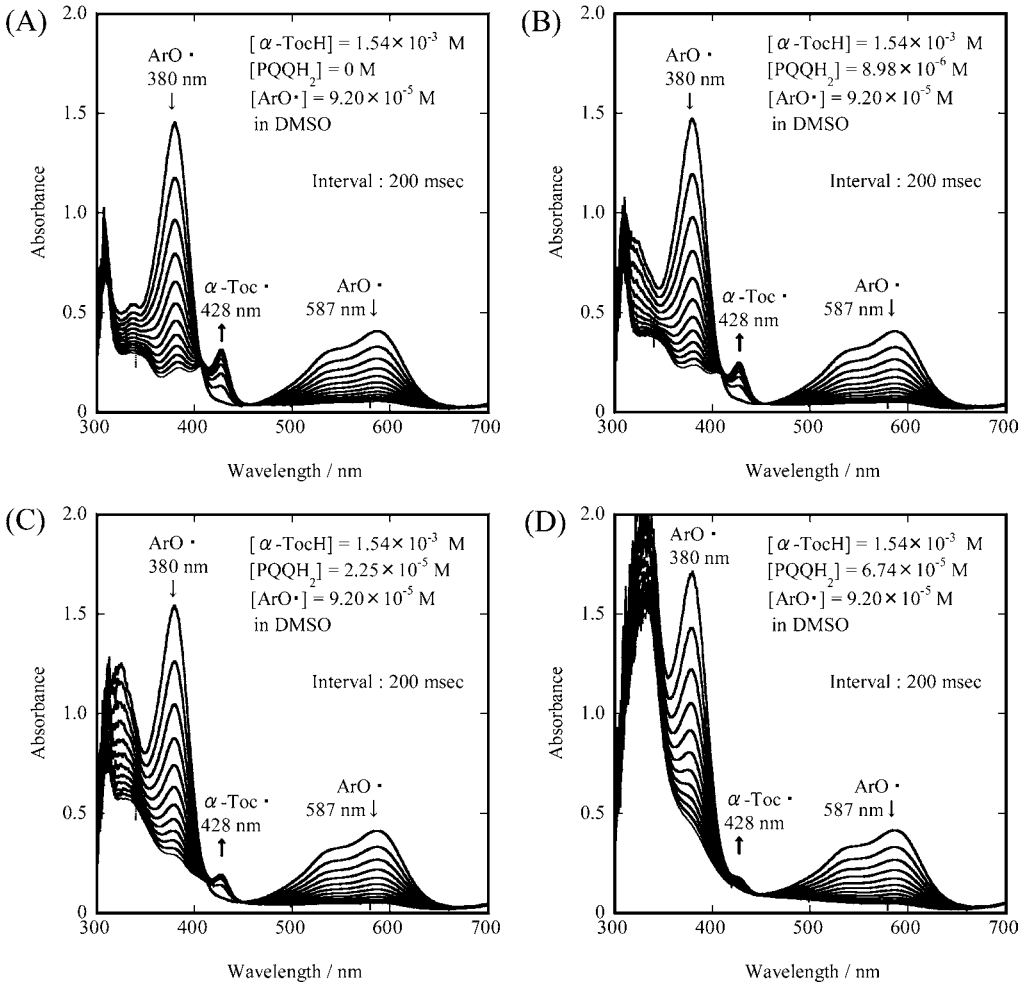

Figure 10. (A) Change in electronic absorption spectra of $ArO^{\bullet}$ and α-$Toc^{\bullet}$ radicals during reaction of $ArO^{\bullet}$ with a mixture of α-TocH and $PQQH_2$ in DMSO at 25.0 °C. $[ArO^{\bullet}] = 9.20 \times 10^{-5}$ and $[\alpha\text{-TocH}] = 1.54 \times 10^{-3}$ M. Spectra were recorded at 200 ms intervals. Absorption of α-$Toc^{\bullet}$ decreased with increasing concentrations of $PQQH_2$. (A) $[PQQH_2] = 0$ M, (B) 8.98×10^{-6} M, (C) 2.25×10^{-5} M, and (D) 6.74×10^{-5} M.

shown in Figure 12B, if $[PQQH_2] = 4.49 \times 10^{-5}$ M (plot i), absorption of α-$Toc^{\bullet}$ completely disappears at ~9 s. As observed for $UQ_{10}H_2$, $PQQH_2$ having two HO groups in a molecule may also quickly regenerate two molecules of α-$Toc^{\bullet}$ to two molecules of α-TocH, because the initial $[\alpha\text{-Toc}^{\bullet}]$ (= $[ArO^{\bullet}]$) is 9.20×10^{-5} M (see Figures 10 and 12). Further, the result suggests that the contribution of NH group in $PQQH_2$ to the regeneration reaction of α-$Toc^{\bullet}$ is negligible. With increasing $[PQQH_2]$ (= (i) 4.49×10^{-5}, (j) 6.74×10^{-5}, (k) 1.80×10^{-4} M), the time at which absorption of α-$Toc^{\bullet}$ disappears decreases in the order of ~9, 5, 2 s, respectively.

DISCUSSION

Comparison of Aroxyl Radical-Scavenging and α-Tocopheroxyl-Regeneration Rates (k_s and k_r) of $PQQH_2$ with the Other Natural Antioxidants in DMSO Solution. α-TocH is well-known as one of the most important lipophilic antioxidants (AOHs) in foods and biological systems.[32−35] The antioxidant action of α-TocH has been ascribed to the scavenging reaction of $LOO^{\bullet}$, producing the corresponding α-$Toc^{\bullet}$ radical (reaction 13).[32] On the other hand, if α-TocH exists in biomembranes and edible oils, α-$Toc^{\bullet}$ radicals may react with unsaturated lipids (LHs) (reaction 14). Reaction 14 is known as a prooxidant reaction, which induces degradation of unsaturated lipids.[36−39]

$$LOO^{\bullet} + \alpha\text{-TocH} \xrightarrow{k_{inh}} LOOH + \alpha\text{-Toc}^{\bullet} \quad (13)$$

$$\alpha\text{-Toc}^{\bullet} + LH \xrightarrow{k_p} \alpha\text{-TocH} + L^{\bullet} \quad (14)$$

$UQ_{10}H_2$ is also well-known as a representative lipophilic AOH.[40,41] $UQ_{10}H_2$ functions as an AOH by (i) scavenging $LOO^{\bullet}$ (reaction 15) and (ii) regenerating α-$Toc^{\bullet}$ to α-TocH (reaction 16),[32,40,41]

$$LOO^{\bullet} + UQ_{10}H_2 \xrightarrow{k_{inh}} LOOH + UQ_{10}H^{\bullet} \quad (15)$$

$$\alpha\text{-Toc}^{\bullet} + UQ_{10}H_2 \xrightarrow{k_r} \alpha\text{-TocH} + UQ_{10}H^{\bullet} \quad (16)$$

where $UQ_{10}H^{\bullet}$ denotes a ubisemiquinone radical. The results of the kinetic studies for reactions 15 and 16 indicated that both reactions are important for the antioxidant actions of $UQ_{10}H_2$.[29,42−44]

On the other hand, Vit C (ascorbate monoanion, AsH^-) is a representative water-soluble AOH. Hydrophilic AsH^- also enhances the antioxidant activity of α-TocH by regenerating α-$Toc^{\bullet}$ to α-TocH (reaction 17).[45−47]

$$\alpha\text{-Toc}^{\bullet} + AsH^- \xrightarrow{k_r} \alpha\text{-TocH} + As^{-\bullet} \quad (17)$$

where $As^{-\bullet}$ is ascorbate free radical.

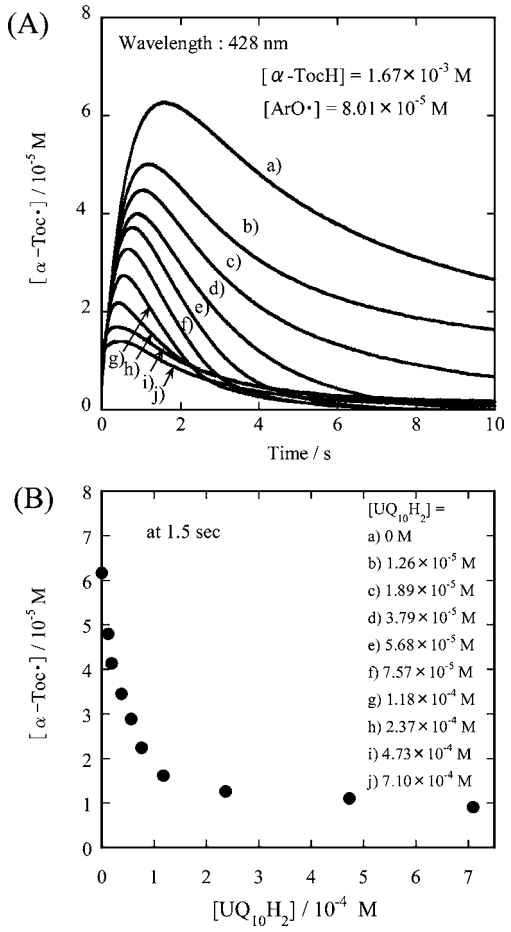

Figure 11. (A) Time dependences of the concentration of α-Toc$^{\bullet}$ radical [α-Toc$^{\bullet}$] (at 428 nm) in DMSO including 11 different concentrations of $UQ_{10}H_2$ at 25.0 °C. (B) [α-Toc$^{\bullet}$] at 1.5 s in panel A versus [$UQ_{10}H_2$] plot.

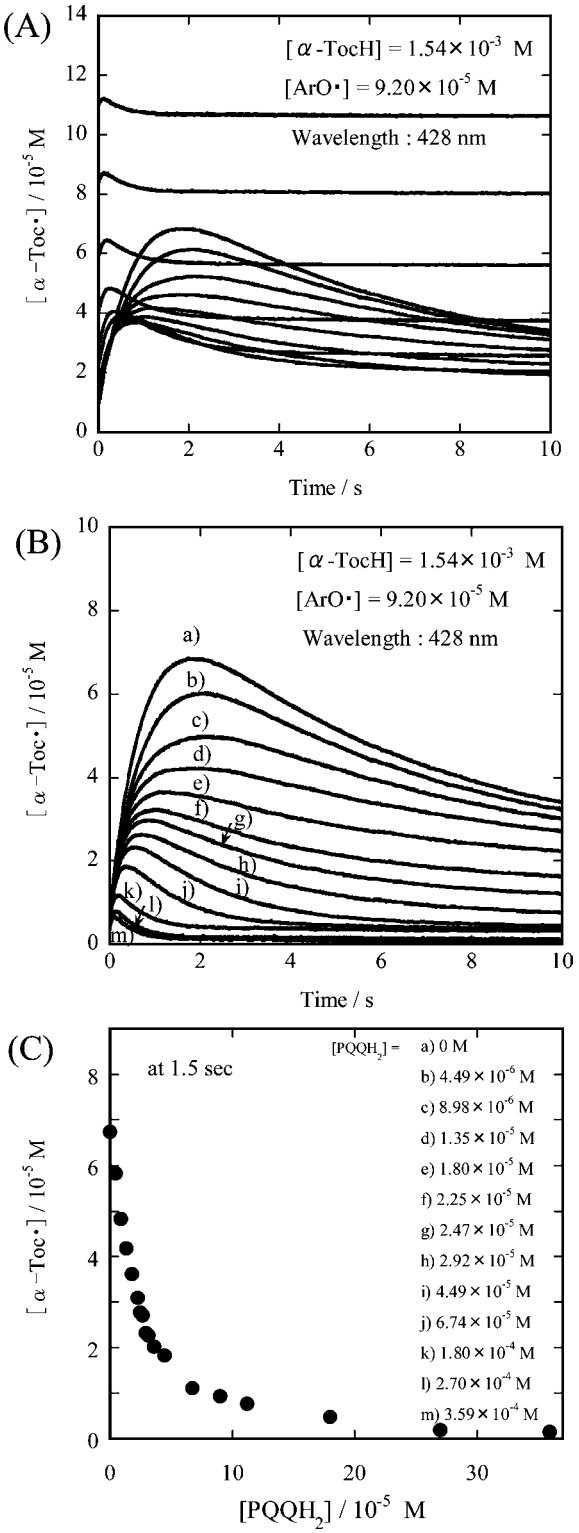

Figure 12. (A) Time dependences of the concentration of α-Toc$^{\bullet}$ radical [α-Toc$^{\bullet}$] (at 428 nm) in DMSO including 13 different concentrations of $PQQH_2$ at 25.0 °C. (B) Baseline corrections for the absorption of $PQQH_2$ in panel A were performed. (C) [α-Toc$^{\bullet}$] at 1.5 s in panel B versus [$PQQH_2$] plot.

In a previous study, we measured the k_s values for reaction of ArO$^{\bullet}$ radical with $PQQH_2$ and water-soluble AOHs (Vit C, cysteine, glutathione, and UA) in 5.0 wt % Triton X-100 micellar solution (pH 7.4).[17] The k_s values decreased in the order of $PQQH_2$ > Vit C ≫ cysteine > UA > glutathione. The k_s value of $PQQH_2$ was 7.4 times larger than that of Vit C.

Furthermore, the k_Q values for reaction of $^1O_2$ with $PQQH_2$, $PQQNa_2$, and seven kinds of water- and lipid-soluble AOHs (Vit C, UA, EC, EGC, α-TocH, $UQ_{10}H_2$, and β-carotene (β-Car)) were measured in micellar solution (pH 7.4).[27] The k_Q values decreased in the order of β-Car > $PQQH_2$ > α-Toc > UA > $UQ_{10}H_2$ > Vit C ≈ EGC > EC ≫ $PQQNa_2$. The $^1O_2$-quenching activity of $PQQH_2$ was found to be 6.3, 2.2, 6.1, and 22 times larger than the corresponding ones of water-soluble AOHs (Vit C, UA, EGC, and EC). Further, the activity of $PQQH_2$ was found to be 2.2 and 3.1 times larger than the corresponding ones of lipid-soluble AOHs (α-Toc and $UQ_{10}H_2$), respectively. On the other hand, the activity of $PQQH_2$ is 6.4 times smaller than that of β-Car. The result suggests that $PQQH_2$ may contribute to the protection of oxidative damage in biological systems, by quenching $^1O_2$.

In the present work, k_s value was measured for $PQQH_2$ and seven AOHs in DMSO solution. As listed in Table 1, the k_s value of $PQQH_2$ decreased in the order of eq 6. The k_s value of $PQQH_2$ was greater than those of water-soluble AOHs (EGCG, EGC, EC, and CA). However, it was less than those of lipid-soluble AOHs (α-TocH and $UQ_{10}H_2$) in DMSO. Further, measurement of k_r value was performed for $PQQH_2$ and $UQ_{10}H_2$ in DMSO solution. The k_r value for $PQQH_2$ was 3.0 times larger than that for $UQ_{10}H_2$, as listed in Table 1.

dx.doi.org/10.1021/jf4040496 | J. Agric. Food Chem. 2013, 61, 11048−11060

Measurements of the k_s and k_r values of Vit C (and Na^+AsH^-) were tried in DMSO to compare with that of $PQQH_2$. However, to our regret, we could not determine the k_s and k_r values, because of low solubility of Vit C (and Na^+AsH^-) in DMSO.

As described above, the k_Q value of $PQQH_2$ was greater than those of α-TocH and $UQ_{10}H_2$ in micellar solution (pH 7.4). Similarly, the k_r value of $PQQH_2$ was greater than that of $UQ_{10}H_2$ in DMSO. On the other hand, the k_s value of $PQQH_2$ was less than those of α-TocH and $UQ_{10}H_2$ in DMSO.

Catechins (EC, ECG, EGC, and EGCG) are catechol (or pyrogallol) derivatives. In previous works, detailed kinetic studies have been performed for the reaction of catechins with $ArO^\bullet$ and 5,7-di-isopropyl-tocopheroxyl (5,7-di-iPr-$Toc^\bullet$) radicals in 5.0 wt % Triton X-100 solution.[48,49] The $ArO^\bullet$ radical-scavenging and 5,7-di-iPr-$Toc^\bullet$ radical-regeneration rates (k_s and k_r) of catechins were constant between pH = 4 and 6, and increased rapidly with increasing pH 6−10 in micellar solution. The increase of the k_s and k_r values was considered to be due to the deprotonation of HO group in catechins. $PQQH_2$ is also a catechol derivative. Consequently, we can expect that both k_s and k_r values of $PQQH_2$ increase with increasing pH value. It will be necessary to measure the pH dependence of the k_s, k_r, and k_Q values of $PQQH_2$ in micellar solution in order to clarify the structure−activity relationship of the antioxidant activity of $PQQH_2$ in solution.

Finding of Synergistic Effect on the Aroxyl Radical-Scavenging Rates (k_s) under the Coexistence of α-Tocopherol and $PQQH_2$ (or Ubiquinol-10). The free radical-scavenging AOHs function not only individually but also synergistically with other AOHs. The most well-known interaction is the one between α-TocH and Vit C.[32,45−47] Hydrophilic Vit C present in the aqueous phase efficiently reduces α-$Toc^\bullet$ radical located within the membranes and lipoproteins to regenerate α-TocH (reaction 17) and to inhibit the initiation of chain reaction induced by α-$Toc^\bullet$ (that is the prooxidant effect of α-TocH) (reaction 14). Similarly, lipophilic $UQ_{10}H_2$ regenerates α-TocH during lipid peroxidation in solution, liposomal membranes, low density protein, and mitochondrial membranes (reaction 16).[29,40,41,43]

It is well-known that various AOHs coexist in many foods and plants,[33] and biological systems.[50−53] However, the examples of measurement of free radical-scavenging rate under the coexistence of two AOHs are very limited, as far as we know.[54] Very recently, it has been found that the $ArO^\bullet$ radical-scavenging rates (k_s) increase notably under the coexistence of the above two AOHs ((i) α-TocH and Vit C and (ii) α-TocH and $UQ_{10}H_2$) in 2-propanol/water (5:1, v/v) solution.[55]

PQQ was found in many kinds of fruits and foods.[3,13,56] Furthermore, the existence of small amounts of free PQQ was found in eight human organs, plasma, and urine and in three rat organs.[11] The result suggests that $PQQH_2$ coexists with α-TocH in many biological systems.

Therefore, in the present work, measurements of $ArO^\bullet$ radical-scavenging rate (k_s^{AOH} (alone)) of AOHs (α-TocH, $PQQH_2$, and $UQ_{10}H_2$) were performed in DMSO solution. The k_s^{AOH} values were measured not only for each AOH, but also for mixtures of two kinds of AOHs ((i) α-TocH and $PQQH_2$ and (ii) α-TocH and $UQ_{10}H_2$). As described in the Results section, a notable synergistic effect was observed for the k_s^{AOH} values. For example, the $k_s^{\alpha\text{-TocH}}$ (+$UQ_{10}H_2$) value (1.26×10^3 M^{-1} s^{-1}) obtained in solution including α-TocH and $UQ_{10}H_2$ was 1.72 times larger than the $k_s^{\alpha\text{-TocH}}$ (alone) value (avg 7.34×10^2 M^{-1} s^{-1}) in solution including only α-TocH (see Tables 1 and 2). The effect of the coexistence of α-TocH and $UQ_{10}H_2$ was more notable for the $k_s^{UQ_{10}H_2}$ (+α-TocH). Especially, at low concentration region of $UQ_{10}H_2$ ($(0-3.55) \times 10^{-4}$ M), $k_s^{UQ_{10}H_2}$ (+α-TocH) (3.08×10^3 M^{-1} s^{-1}) was 2.50 times larger than the $k_s^{UQ_{10}H_2}$ (alone) (avg 1.23×10^3 M^{-1} s^{-1}).

Furthermore, the $k_s^{\alpha\text{-TocH}}$ (+$PQQH_2$) (8.26×10^2 M^{-1} s^{-1}) was 1.13 times larger than the $k_s^{\alpha\text{-TocH}}$ (alone) (avg 7.34×10^2 M^{-1} s^{-1}; see Table 1). Similarly, the $k_s^{PQQH_2}$ (+α-TocH) (6.10×10^2 M^{-1} s^{-1}) was 2.42 times larger than the $k_s^{PQQH_2}$ (alone) (avg 2.52×10^2 M^{-1} s^{-1}).

As described above, notable increase of the $ArO^\bullet$ radical-scavenging rate (k_s^{AOH}) was observed for the AOHs under the coexistence of AOHs. However, the mechanism why the k_s^{AOH} values increase under the coexistence of two AOHs is not clear at present.[55]

UV−Vis Absorption of α-Tocopheroxyl Radical Disappears under the Coexistence of α-Tocopherol and $PQQH_2$ (or Ubiquinol-10): Suppression of Prooxidant Effect of α-Tocopherol. As described above, α-$Toc^\bullet$ is an important key radical, which appears in the process of the antioxidant and prooxidant actions of α-TocH (see reactions 13−17). As described in the Results section, formation of α-$Toc^\bullet$ radical was suppressed remarkably under the coexistence of α-TocH and $PQQH_2$ (or $UQ_{10}H_2$). As shown in Figures 8 and 10−12, we could directly ascertain that α-$Toc^\bullet$ radical produced by the reaction with $ArO^\bullet$ radical immediately disappears through the regeneration reaction with $PQQH_2$ and $UQ_{10}H_2$, by observing the decrease of UV−vis absorption of α-$Toc^\bullet$ radical. Furtheremore, it has been clarified that $PQQH_2$ or $UQ_{10}H_2$ having two OH groups within a molecule may rapidly regenerate two molecules of α-$Toc^\bullet$ to two molecules of α-TocH. In fact, the regeneration rate constants (k_r) obtained for $PQQH_2$ and $UQ_{10}H_2$ were very fast, as listed in Table 1. An example for such a direct observation of the disappearance of α-$Toc^\bullet$ radical under the coexistence of α-TocH and $PQQH_2$ (or $UQ_{10}H_2$) has not been reported, as far as we know.

As described above, α-TocH, $PQQH_2$, and $UQ_{10}H_2$ coexist in foods and biological systems (such as plasma, blood, and various tissues).[3,11,13,56] Consequently, the above synergistic effect, that is, the increase of the free radical-scavenging rate and the suppression of the prooxidant reaction, may function in foods and biological systems.

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Funding

This work was partly supported by a Grant-in-Aid for Challenging Exploratory Research (No. 24658123) from the Japan Society for the Promotion of Science.

Notes

The authors declare no competing financial interest.

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