Kinetic Study of the Tocopherol Regeneration Reaction by Biological Hydroquinones in Micellar Solution

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The rate constants (k_r) of the regeneration reaction of 7-*t*-butyl-5-isopropyltocopheroxyl with ubiquinol-10 ($UQ_{10}H_2$), ubiquinol-0 (UQ_0H_2), α-, *β*-, and *γ*-tocopherolhydroquinones (α-, *β*-, *γ*-TQH_2), 2,3,5-trimethyl-1,4-hydroquinone ($TMQH_2$), vitamin K_3 hydroquinone (VK_3H_2), and vitamin C (Vit C) have been measured in 2-propanol/water and micellar solutions by a stopped-flow spectrophotometer. The k_r values of these hydroquinones (HQs) in micellar solution remained constant at pHs of 6−9 and increased rapidly by increasing the pH value. The k_r values decreased in the order of VK_3H_2 > *γ*-TQH_2 ≥ α-TQH_2 > *β*-TQH_2 ≥ $UQ_{10}H_2$ ≥ $TMQH_2$ > UQ_0H_2 >> Vit C at pHs of 6−9. These HQs are dibasic acids and can exist in three different molecular forms, depending on pH. By comparing the k_r values with the mole fraction of each molecular form of the HQs, the reaction rate k_{r1} for the undissociated form, k_{r2} for the monoanion, and k_{r3} for the dianion and the pK_{a1} and pK_{a2} values were determined. It has been found that the k_r values of $UQ_{10}H_2$, α-TQH_2, *β*-TQH_2, and *γ*-TQH_2 (plastoquinol model) are 460, 1430, 494, and 1530 times larger than that of Vit C at pH 7.0, respectively, although the values are similar to that of Vit C in 2-propanol/water. The biological HQs and Vit C coexist in many tissues of animals and plants, and thus, the relative antioxidant activities of HQs and Vit C have been tentatively discussed based on the products of k_r values by concentrations in several tissues. The results suggest that these HQs show high activity for the tocopherol regeneration in biological systems.

1. Introduction

Lipophilic vitamin E (α-tocopherol, α-TocH) is localized in cellular membranes and functions as an antioxidant by protecting unsaturated lipids from peroxidation. The antioxidant properties of the α-TocH have been ascribed to the initial oxidation of the phenolic hydroxyl group by a lipid peroxyl radical (LOO•), producing a α-tocopheroxyl radical (α-Toc•) (reaction 1). The mechanism involved has been studied extensively by several investigators[1−4]

$$\text{LOO•} + \alpha\text{-TocH} \xrightarrow{k_{inh}} \text{LOOH} + \alpha\text{-Toc•} \qquad (1)$$

On the other hand, vitamin C (Vit C) (ascorbate monoanion, AsH^-) is well-known as a water-soluble antioxidant. Hydrophilic vitamin C enhances the antioxidant activity of α-TocH by regenerating α-Toc• to α-TocH (reaction 2)[2,3,5]

$$\alpha\text{-Toc•} + AsH^- \xrightarrow{k_r} \alpha\text{-TocH} + As^-\text{•} \qquad (2)$$

where As^-• is an ascorbate free radical. The mixtures of α-TocH and Vit C may function synergistically as antioxidants in various tissues.[2,5]

Ubiquinone (UQ), vitamin K (VK), and plastoquinone (PQ) are well-known as typical biological quinone compounds. The function common to these quinones in biology is to act as redox components, transferring electrons between protein complexes and protons across the membranes. Ubiquinol (UQH_2), vitamin K hydroquinone (VKH_2), and plastoquinol (PQH_2) (Figure 1) are the two-electron reduction products of UQ, VK, and PQ, respectively.

Ubiquinol-10 ($UQ_{10}H_2$) is also well-known as a representative lipophilic antioxidant.[6,7] α-TocH and $UQ_{10}H_2$ coexist in relatively high concentrations in the plasma and mitochondria of various tissues (heart, liver, kidney, brain, and muscle).[7−13] It has been reported that $UQ_{10}H_2$ functions as an antioxidant (i) by scavenging LOO• (reaction 3) and (ii) by regenerating Toc• to TocH (reaction 4)[14−22]

$$\text{LOO•} + UQ_{10}H_2 \xrightarrow{k_{inh}} \text{LOOH} + UQ_{10}\text{H•} \qquad (3)$$

$$\text{Toc•} + UQ_{10}H_2 \xrightarrow{k_r} \text{TocH} + UQ_{10}\text{H•} \qquad (4)$$

where UQ_{10}H• is the dehydroubiquinol radical. Kinetic studies have been performed for reactions 3 and 4 in organic solvents using chemiluminescense,[23] stopped-flow spectrophotometry,[24,25] and the O_2 consumption method.[26] The results indicated that both reactions are important for the antioxidant actions of $UQ_{10}H_2$. We can expect similar functions for plastoquinol-9 (PQ_9H_2) and vitamin K_1 hydroquinone (VK_1H_2). α-Tocopherolquinone (α-TQ) and α-TocH are natural components of the photosynthetic membranes occurring at about 10 and 25−30% of the amount of plastoquinone-9 (PQ_9), respectively.[27] It has been reported that α-tocopherolhydroquinone (α-TQH_2) is an efficient multifunctional inhibitor of the radical-initiated oxidation of low-density lipoprotein lipids.[28] However, the details of the antioxidant activity of these HQs in biological systems have not been clarified.

In a previous work, the rate constants (k_r) of the regeneration reaction of α-TocH with biological hydroquinones (HQs) **1**−**7**

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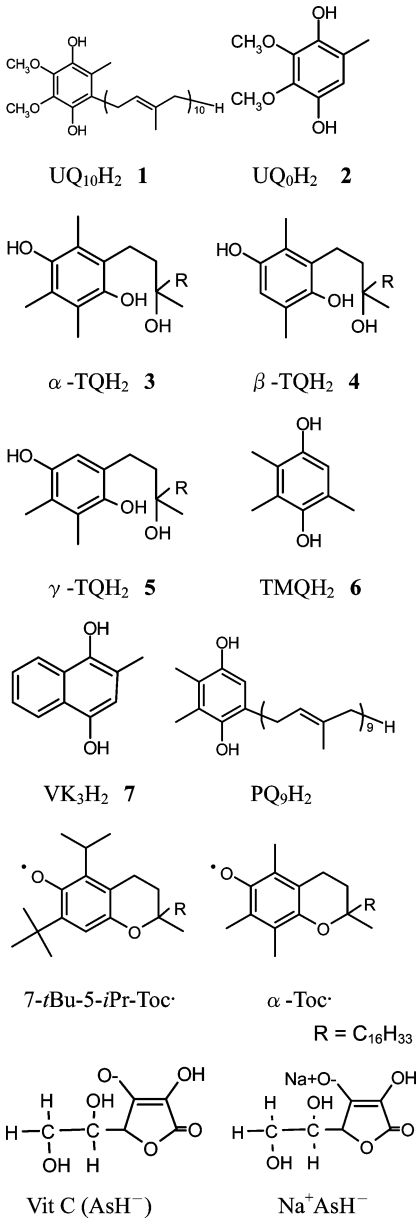

Figure 1. Molecular structures of ubiquinol-10 ($UQ_{10}H_2$ **1**), ubiquinol-0 (UQ_0H_2 **2**), α-, *β*-, and *γ*-tocopherol hydroquinone (α-, *β*-, and *γ*-TQH_2 **3**, **4**, and **5**), trimethylhydroquinone ($TMQH_2$ **6**), vitamin K_3 (VK_3H_2 **7**), plastoquinol-9 (PQ_9H_2), the 7-*tert*-butyl-5-isopropyltocopheroxyl (7-*t*Bu-5-iPr-Toc•) radical, the α-tocopheroxyl (α-Toc•) radical, vitamin C (ascorbate monoanion, AsH^-), and sodium ascorbate (Na^+AsH^-).

(ubiquinol-10 ($UQ_{10}H_2$), ubiquinol-0 (UQ_0H_2), α-, *β*-, and *γ*-tocopherolhydroquinones (α-, *β*-, *γ*-TQH_2), 2,3,5-trimethyl-1,4-hydroquinone ($TMQH_2$), and vitamin K_3 hydroquinone (VK_3H_2)) (see Figure 1) were measured in 2-propanol/water (5:1, v/v) mixtures, showing fast tocopherol regeneration rates (see Table 2 in ref 25). We tried to measure the reaction rates (k_r) between α-Toc• and the above biological HQs in micellar solution. However, we were unsuccessful in measuring the rate constants because α-Toc• is unstable and disappears rapidly by bimolecular reaction in micellar solution. Further, the reaction rates between α-Toc• and HQs in micellar solution were too fast to be determined.[25]

In the present work, in order to clarify the structure−activity relationship in the regeneration reaction of the tocopheroxyl radical by biological HQs, we have measured the second-order

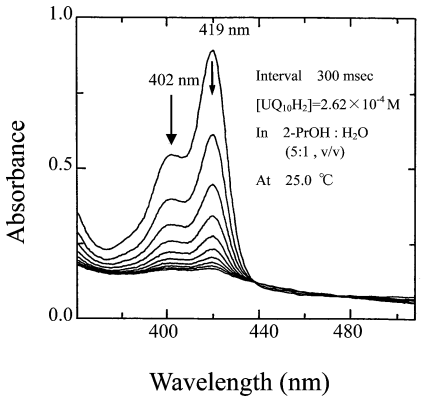

Figure 2. Change in the electronic absorption spectrum of the 7-*tert*-butyl-5-isopropyltocopheroxyl radical (Toc•) during reaction of Toc• with ubiquinol-10 in a 2-propanol/water (5:1, v/v) solution at 25.0 °C; $[\text{ubiquinol-10}]_{t=0} = 0.262$ mM. The spectra were recorded at 300 ms intervals. The arrow indicates a decrease in absorbance with time.

rate constants (k_r) for the reaction of the above HQs and Vit C with the 7-*tert*-butyl-5-isopropyltocopheroxyl (7-*t*Bu-5-iPr-Toc•) radical in 2-propanol/water (5:1, v/v) and aqueous Triton X-100 (5.0 wt %) micellar solutions (reaction 4), where *γ*-tocopherol-hydroquinone (*γ*-TQH_2) is considered to be a model of plastoquinol-9 (PQ_9H_2) (see Figure 1).[25] The 7-*t*Bu-5-iPr-Toc• radical is stable because it has bulky *tert*-butyl and isopropyl substituents at the 7 and 5 positions, respectively. The rate constants (k_r) obtained in micellar solution were pH-dependent because of the dissociation of two phenolic hydroxyl groups in the hydroquinone molecules.[29,30] The α-TocH, Vit C, and these biological HQs coexist in the various tissues of animals and plants. Therefore, the relative rates of tocopheroxyl regeneration reactions ($-\text{d[Toc•]}/\text{d}t$), that is, the antioxidant activities of these HQs, have been tentatively discussed based on the rate constants (k_r) obtained and their concentrations in biological systems. Recently, it has been reported that the rate constants (k_r) of the tocopherol regeneration reaction with Vit C, catechins, and flavone derivatives show notable pH dependence in micellar solutions.[31−34] However, the pH dependence of the rate constants (k_r) for these HQs has not been reported.

2. Experimental Methods

2.1. Materials. 2,3,5-Trimethyl-1,4-hydroquinone and vitamin K_3 are commercially available. Ubiquinone-10 and ubiquinone-0 were kindly supplied by Kaneka Co. Ltd. and Taoka Co. Ltd., respectively. The α-, *β*-, and *γ*-tocopherolquinones were prepared by the oxidation of the corresponding tocopherols in diethyl ether with $FeCl_3 \cdot 6H_2O$ in a CH_3OH/H_2O (1:1, v/v) solution.[35] Ubiquinol-10, ubiquinol-0, α-, *β*-, and *γ*-tocopherol-hydroquinones, and vitamin K_3 hydroquinone were prepared by the reduction of the corresponding quinones with sodium hydrosulfite in *n*-hexane (or in *n*-hexane/ethanol) under a nitrogen atmosphere. 7-*tert*-Butyl-5-isopropyltocopherol (7-*t*Bu-5-iPr-TocH) was prepared according to the method reported in a previous paper.[31,36] The 7-*t*Bu-5-iPr-Toc• radical is fairly stable and was prepared by the PbO_2 oxidation of the corresponding tocopherol in a 2-propanol/water (5:1, v/v) solution under a nitrogen atmosphere. In the case of the reaction in micellar solution, 7-*t*Bu-5-iPr-Toc• was prepared by the reaction between the 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl (ArO•) radical[37] and 7-*t*Bu-5-iPr-TocH in an aqueous Triton X-100 micellar solution (5.0 wt %) at 25 °C and was reacted immediately with a Triton X-100 micellar solution (5.0 wt %) of the HQs **1**−**7**.[31,34]

TABLE 1: The Second-Order Rate Constants (k_r) for the Reaction of Biological Hydroquinones (HQs 1−7) and Vitamin C with the 7-*t*Bu-5-iPr-Toc• Radical in 2-Propanol/Water (5:1, v/v) and in Triton X-100 Micellar (5.0 wt %) Solutions at 25.0 °C, the Relative Rate Constants (k_r(HQ)/k_r($UQ_{10}H_2$)), and Peak Oxidation Potentials (E_P)

	7-*t*Bu-5-iPr-Toc•		7-*t*Bu-5-iPr-Toc•			
	2-PrOH/H_2O (5:1, v/v) $k_r/M^{-1}\,s^{-1}$	ratio k_r(HQ)/k_r($UQ_{10}H_2$)	Triton X-100 micelle $k_r/M^{-1}\,s^{-1}$ (pH 7.0)	ratio k_r(HQ)/k_r($UQ_{10}H_2$)	ratio k_r(micelle)/k_r(2-PrOH/H_2O)	CH_3CN E_p/mV vs SCE
$UQ_{10}H_2$ **1**	7.54×10^3	1.00	1.48×10^5	1.00	19.6	930
UQ_0H_2 **2**	1.88×10^3	0.249	1.24×10^5	0.841	66.0	960
α-TQH_2 **3**	1.65×10^4	2.19	4.62×10^5	3.12	28.0	830
β-TQH_2 **4**	1.09×10^4	1.44	1.59×10^5	1.07	14.6	870
γ-TQH_2 **5** (PQ_9H_2 model)	1.47×10^4	1.95	4.93×10^5	3.33	33.5	880
$TMQH_2$ **6**	8.79×10^3	1.16	1.43×10^5	0.966	16.2	870
VK_3H_2 **7**	1.08×10^5	14.3	2.95×10^6	19.9	27.3	770
Vit C (AsH^-)			3.22×10^2 [a]	2.18×10^{-3}	1/23.4	
Vit C (Na^+AsH^-)	7.53×10^3	1.00	3.00×10^2 [a]	2.03×10^{-3}	1/25.1	

[a] See ref 31.

All buffer solutions were prepared using distilled water treated with a Millipor Q system. The pH of the solution was adjusted using an appropriate buffer (0.1 M), $KH_2PO_4-Na_2HPO_4$ at pHs of 6−9.5 and $NaHCO_3-Na_2CO_3$ at pHs of 9.75−11.5.

2.2. Measurements. The kinetic data were obtained with a Unisoku Model RS-450 stopped-flow spectrophotometer by mixing equal volumes of solutions of HQs and 7-*t*Bu-5-iPr-Toc• under a nitrogen atmosphere.[31,34] All measurements were performed at 25.0 ± 0.5 °C. Experimental errors in the rate constants (k_r) were estimated to be about 5 and 8% in homogeneous and micellar solutions, respectively.

3. Results

3.1. Rate Constants (k_r) of the Tocopherol Regeneration Reaction with Biological Hydroquinones 1−7 in a 2-Propanol/Water Solution. Measurement of the rate constant (k_r) for the reaction of the 7-*t*Bu-5-iPr-Toc• radical with $UQ_{10}H_2$ was performed in a homogeneous 2-propanol/water (5:1, v/v) solution. By reacting $UQ_{10}H_2$ with the Toc• radical, the absorbance at 419 and 402 nm of the Toc• decreased rapidly, as shown in Figure 2. The rate was measured by following the decrease in absorbance at 419 nm of the Toc• radical, as described in previous works.[24,25,31] The pseudo-first-order rate constants (k_{obsd}) obtained were linearly dependent on the concentration of $UQ_{10}H_2$ ([$UQ_{10}H_2$]), and thus, the rate equation is expressed as

$$-\mathrm{d[Toc\bullet]}/\mathrm{d}t = k_{obsd}[\mathrm{Toc\bullet}] = k_r[\mathrm{UQ_{10}H_2}][\mathrm{Toc\bullet}] \quad (5)$$

where k_r is the second-order rate constant for oxidation of $UQ_{10}H_2$ by the Toc• radical. The rate constants ($k_r = 7.54 \times 10^3\ M^{-1}\,s^{-1}$) were obtained by plotting k_{obsd} against [$UQ_{10}H_2$].

Similar measurements were performed for the reactions of 7-*t*Bu-5-iPr-Toc• with six kinds of biological HQs **2**−**7** (UQ_0H_2, α-, *β*-, and *γ*-TQH_2, $TMQH_2$, and VK_3H_2) and sodium ascorbate (Na^+AsH^-) in a 2-propanol/water solution. The k_r values obtained are summarized in Table 1.

As is clear from the k_r values listed in Table 1, the rate constants of the regeneration reaction of Toc• with the above biological HQs **1**−**7** and Na^+AsH^- decreases in the order of

$$VK_3H_2 > \alpha\text{-}TQH_2 > \gamma\text{-}TQH_2 > \beta\text{-}TQH_2 > TMQH_2 > UQ_{10}H_2 \sim Na^+AsH^- > UQ_0H_2 \quad (6)$$

The rate constants of α-, *β*-, and *γ*-TQH_2, $TMQH_2$, and VK_3H_2 are 2.2, 1.4, 2.0, 1.2, and 14 times as large as that of $UQ_{10}H_2$, respectively, in 2-propanol/water. The rate constant of $UQ_{10}H_2$ is very similar to that of Na^+AsH^- (Vit C) in homogeneous solution.

3.2. pH Dependence of the Rate Constants (k_r) of the Tocopherol Regeneration Reaction with Biological Hydroquinones 1−7 in Micellar Solution. Measurement of the rate constant (k_r) for the reaction of the 7-*t*Bu-5-iPr-Toc• radical with $UQ_{10}H_2$ was performed at various pH values in a 5.0 wt % Triton X-100 micellar solution. By reacting the Toc• radical with $UQ_{10}H_2$, the absorbance at 418 and 399 nm of the Toc• decreased. The rate was measured by following the decrease in absorbance at 418 nm of the Toc• radical.[25,31] The k_{obsd} versus [$UQ_{10}H_2$] plots (eq 5) at pH = 7.0, 9.0, 10.5, 11.0, and 11.5 are shown in Figure 3. As shown in Figure 4a, the rate constants (k_r) of $UQ_{10}H_2$ are pH-independent and show similar values at pH values of 6−9 and then increase rapidly at about pH 10.

Similar measurements were performed for the reactions of Toc• with α-, *β*-, and *γ*-TQH_2, and $TMQH_2$ in micellar solution by varying pH values. The reaction rates of UQ_0H_2 and VK_3H_2 were measured only at pH = 7.0. The k_r values obtained are summarized in Tables 1 and 2, together with that reported for Vit C.[31] The rate constants (k_r) of these HQs remain constant in the low pH region and increase rapidly at about pH 10, as shown in Figure 4b−e. The values of $UQ_{10}H_2$, α-, *β*-, and *γ*-TQH_2, and $TMQH_2$ at pH 11.0 are 2.1, 2.6, 2.3, 2.2, and 3.4

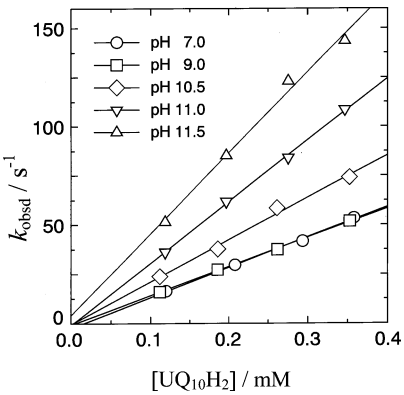

Figure 3. Dependence of pseudo-first-order rate constants (k_{obsd}) on concentration of ubiquinol-10 at pH = 7.0, 9.0, 10.5, 11.0, and 11.5 in a 5.0 wt % Triton X-100 micellar solution.

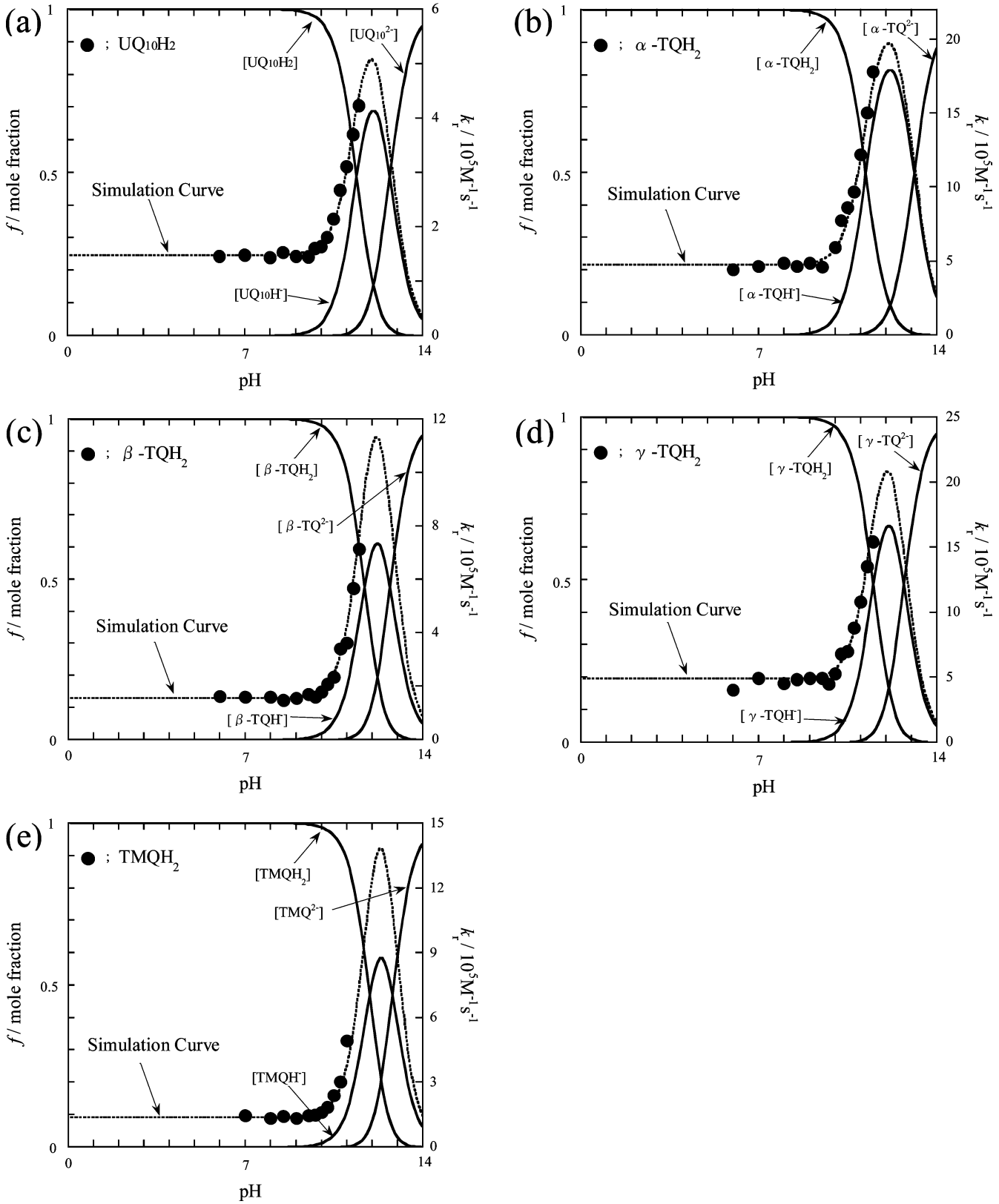

Figure 4. (a) Plots of second-order rate constant (k_r) for ubiquinol-10 ($UQ_{10}H_2$) versus pH (closed circle) and of the mole fraction (f) of three ubiquinol-10 species ($UQ_{10}H_2$, $UQ_{10}H^-$, and UQ_{10}^{2-}) versus pH (solid line). The dotted line is a simulation curve. (b−e) Similar plots for α-, β-, and γ-tocopherolhydroquinones (α-, β-, and γ-TQH_2) and 2,3,5-trimethylhydroquinone ($TMQH_2$).

times as large as those at pH 7.0, respectively. The pH dependence of the reaction rates observed will be discussed in a later section.

As listed in Tables 1 and 2, the rate constants of the regeneration reaction of Toc• with the above biological HQs and Vit C (AsH^-) at pHs of 7−9 decrease in the order of

$$VK_3H_2 > \gamma\text{-}TQH_2 \geq \alpha\text{-}TQH_2 > \beta\text{-}TQH_2 \geq UQ_{10}H_2 \sim TMQH_2 > UQ_0H_2 >> AsH^- \quad (7)$$

The rate constants of α-, β-, and γ-TQH_2 and VK_3H_2 at pH 7.0 are 3.1, 1.1, 3.3, and 20 times as large as that of $UQ_{10}H_2$,

TABLE 2: pH Dependence of the Second-Order Rate Constants (k_r) for the Reaction of Biological Hydroquinones with the 7-*t*-Butyl-5-isopropyltocopheroxyl (7-*t*Bu-5-iPr-Toc•) Radical in a Triton X-100 Micellar Solution (5.0 wt %) at 25.0 °C

	$k_r/M^{-1}\,s^{-1}$				
pH	$UQ_{10}H_2$	α-TQH_2	β-TQH_2	γ-TQH_2	$TMQH_2$
6.00	1.46×10^5	4.43×10^5	1.61×10^5	4.01×10^5	
7.00	1.48×10^5	4.62×10^5	1.59×10^5	4.93×10^5	1.43×10^5
8.00	1.43×10^5	4.84×10^5	1.58×10^5	4.49×10^5	1.32×10^5
8.50	1.53×10^5	4.63×10^5	1.47×10^5	4.81×10^5	1.42×10^5
9.00	1.46×10^5	4.87×10^5	1.54×10^5	4.94×10^5	1.31×10^5
9.50	1.44×10^5	4.59×10^5	1.67×10^5	4.89×10^5	1.44×10^5
9.75	1.60×10^5		1.58×10^5	4.46×10^5	1.47×10^5
10.00	1.64×10^5	5.91×10^5	1.78×10^5	5.25×10^5	1.58×10^5
10.25	1.81×10^5	7.72×10^5	2.08×10^5	6.77×10^5	1.84×10^5
10.50	2.14×10^5	8.59×10^5	2.33×10^5	6.96×10^5	2.38×10^5
10.75	2.67×10^5	9.68×10^5	3.39×10^5	8.80×10^5	3.01×10^5
11.00	3.11×10^5	1.22×10^6	3.62×10^5	1.08×10^6	4.90×10^5
11.25	3.70×10^5	1.50×10^6	5.67×10^5	1.35×10^6	
11.50	4.23×10^5	1.78×10^6	7.14×10^5	1.54×10^6	

respectively, in micellar solution. The order of the rates (k_r) in micellar solution is similar to that in 2-propanol/water, although the rates of HQs **1**−**7** in micellar solution are 15−66 times larger than the corresponding rates in 2-propanol/water, except for Vit C. In the case of Vit C, the rate ($k_r = 3.22 \times 10^2\ M^{-1}\ s^{-1}$) in micellar solution is 23 times smaller than that ($k_r = 7.53 \times 10^3\ M^{-1}\ s^{-1}$) in 2-propanol/water.

The oxidation potentials (E_p) for these HQs **1**−**7** were reported in a previous work (see Table 1).[25] The log k_r was plotted against E_p. As shown in Figure 5, similar linear correlations between log k_r and E_p were observed for the HQs **1**−**7** in 2-propanol/water (5:1, v/v) and micellar solutions with gradients of −7.91/V and −6.77/V (correlation coefficients = 0.941 and 0.849), respectively. The rate constants (k_r) of the regeneration reaction of the Toc• radical with biological HQs in solutions increase as the electron-donating capacity of the biological HQs increases.[25,36,38]

As listed in Table 1 and as shown in Figure 5, the k_r (and E_p) values for β-TQH_2, γ-TQH_2, and $TMQH_2$ are similar to each other in the 2-propanol/water solution because these HQs have three alkyl substituents on the aromatic ring. α-TQH_2, having four alkyl substituents, shows larger k_r and smaller E_p values than β-TQH_2, γ-TQH_2, and $TMQH_2$. PQ_9H_2, which is very important as an electron carrier in photosynthetic systems, also has three alkyl substituents on the aromatic ring, and we can expect that the reaction rate of PQ_9H_2 is similar to those of β-TQH_2, γ-TQH_2, and $TMQH_2$. In particular, both PQ_9H_2 and γ-TQH_2 have two methyl substituents at positions 2 and 3 and a long alkyl chain at position 6. Consequently, γ-TQH_2 is considered to be a model of PQ_9H_2. In micelle solution, the k_r values of β-TQH_2 and $TMQH_2$ are similar to each other and are three times smaller than that of α-TQH_2, as expected. On the other hand, the k_r value of γ-TQH_2 (PQ_9H_2 model) is similar to that of α-TQH_2. The reason is not clear at present. It may be due to the difference in the polarity of the reaction field because the reaction rates change remarkably depending on the polarity of the reaction field, as described in the following section.[25,39,40]

α-Tocopherolquinone (α-TQ) is a natural component of the photosynthetic membranes occurring at about 10% of the amount of plastoquinone-9 (PQ_9).[27] The results of the present kinetic study suggest that α-TQH_2 and PQ_9H_2 also have high activity for the tocopherol regeneration and contribute to the prevention of lipid peroxidation in biological systems.

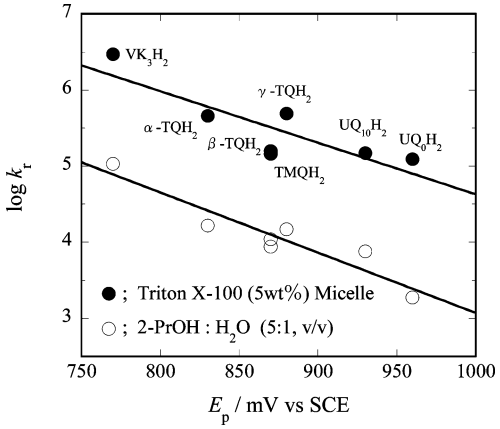

Figure 5. Plots of log k_r for the reaction of biological hydroquinones (HQs) with the 7-*tert*-butyl-5-isopropyltocopheroxyl radical in 2-propanol/water (5:1, v/v) (open circle) and micellar solutions (closed circle) versus E_p.

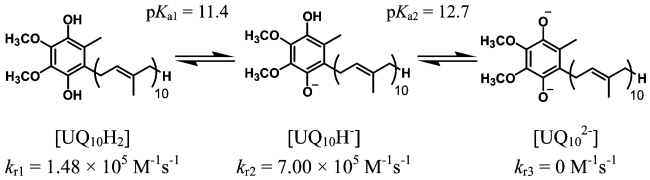

Figure 6. Three different molecular forms of ubiquinol-10 ($UQ_{10}H_2$, $UQ_{10}H^-$, and UQ_{10}^{2-}) in micellar solution and their reaction rates, k_{ri}.

4. Discussion

4.1. Analyses of the pH Dependence on the Reaction Rates (k_r) of Biological Hydroquinones 1 and 3−6. Ubiquinol-10 ($UQ_{10}H_2$) is dibasic and can exist in three different molecular forms, that is, the undissociated form ($UQ_{10}H_2$), the monoanion ($UQ_{10}H^-$), and the dianion (UQ_{10}^{2-}), depending on the pH value (see Figure 6). The equilibrium reactions of ubiquinol-10 have the form

$$UQ_{10}H_2 \overset{K_{a1}}{\rightleftharpoons} UQ_{10}H^- \overset{K_{a2}}{\rightleftharpoons} UQ_{10}^{2-} \tag{8}$$

If the pK_{a1} and pK_{a2} values of ubiquinol-10 are reported, the mole fractions (f) present as the $UQ_{10}H_2$ molecule and the $UQ_{10}H^-$ and UQ_{10}^{2-} ions may be calculated as a function of pH (see Figure 4a).[31−34] The analytical concentration (C_a) is given as

$$C_a = [UQ_{10}H_2] + [UQ_{10}H^-] + [UQ_{10}^{2-}] \tag{9}$$

If we assume that the k_{r1}, k_{r2}, and k_{r3} are the reaction rates for $UQ_{10}H_2$, $UQ_{10}H^-$, and UQ_{10}^{2-} forms of ubiquinol-10, respectively, the total rate k_r will be expressed as

$$k_r = k_{r1}\, f(UQ_{10}H_2) + k_{r2}\, f(UQ_{10}H^-) + k_{r3}\, f(UQ_{10}^{2-}) \tag{10}$$

At pH = 6−9, only the undissociated form of ubiquinol-10 exists in solution, that is, $f(UQ_{10}H_2) = 1$, and we can immediately determine the k_{r1} value. The k_{r3} value is considered to be small compared to k_{r2} and is negligible because the dianion form (UQ_{10}^{2-}) of ubiquinol-10 does not have any OH proton to reduce Toc•.[41] Therefore, by comparing the observed pH dependence of k_r with the pH dependence of the mole fraction calculated, the values of pK_{a1}, pK_{a2}, and k_{r2} were determined. The k_{r1}, k_{r2}, and k_{r3} values obtained are listed in Table 3. As shown in Figure 4a, a good accordance between the observed k_r and simulation curve was obtained, suggesting that each

TABLE 3: The Reaction Rates (k_{ri}, $i = 1-3$) for the Undissociated, Monoanion, and Dianion Forms and pK_{ai} ($i = 1$ and 2) Values of Five Kinds of Biological Hydroquinones and Vitamin C (AsH_2) in a Triton X-100 Micellar Solution (5.0 wt %) at 25.0 °C

antioxidant	$k_{r1}/M^{-1}\,s^{-1}$	$k_{r2}/M^{-1}\,s^{-1}$	$k_{r3}/M^{-1}\,s^{-1}$	k_{r2}/k_{r1}	pK_{a1}[a]	pK_{a2}[a]
$UQ_{10}H_2$	1.48×10^5	7.00×10^5	0	4.7	11.4 (11.3[b])	12.7 (13.2[b])
α-TQH_2	4.74×10^5	2.36×10^6	0	5.0	11.2	13.1
β-TQH_2	1.56×10^5	1.80×10^6	0	11.5	11.7	12.7
γ-TQH_2	4.89×10^5	3.00×10^6	0	6.1	11.5	12.7
$TMQH_2$	1.37×10^5	2.32×10^6	0	16.9	11.9 (10.8[c])	12.8 (12.9[c])
AsH_2	−	3.19×10^2	−		4.17	11.57

[a] Experimental errors in pK_{a1} and pK_{a2} values are estimated to be about ±0.1 and ±0.5, respectively. [b] See ref 29. [c] See ref 30.

reaction rate (k_{ri}) and pK_{ai} value estimated are reasonable. The pK_{a1} (=11.4) and pK_{a2} (=12.7) values obtained in the present work are similar to those (pK_{a1} = 11.3 and pK_{a2} = 13.2) reported by Rich,[29] as listed in Table 3.

The α-, *β*-, and *γ*-TQH_2 and $TMQH_2$ are also dibasic. The reaction rates k_{r1}, k_{r2}, and k_{r3} for three different molecular forms were determined similarly, by assuming that the k_{r3} value for the dianion (α-, *β*-, and *γ*-TQ^{2-} and TMQ^{2-}) is small and negligible.[41] The pK_{a1} and pK_{a2} values of α-, *β*-, and *γ*-TQH_2 have not been reported, as far as we know. The values of pK_{a1} (=11.2−11.9) and pK_{a2} (=12.7−13.1) obtained for these HQs are similar to one another, as listed in Table 3. The k_{r2} values for the monoanion ($UQ_{10}H^-$, α-, *β*-, and *γ*-TQH^-, and $TMQH^-$) are 4.7, 5.0, 11.5, 6.1, and 16.9 times as large as the k_{r1} values for the undissociated form ($UQ_{10}H_2$, α-, *β*-, and *γ*-TQH_2, and $TMQH_2$), respectively. The oxidation potentials of the monoanion form of the HQs are smaller than the corresponding ones of the reduced form, and thus, the k_{r2} values will increase.[36,38]

4.2. Effect of the Reaction Field on the Reaction Rates (k_r) of Biological Hydroquinones 1−7 and Vitamin C. The solvent effects on the reaction rates of 5,7-di-isopropyltocopheroxyl (5,7-Di-iPr-Toc•) (k_r) and ArO• (k_s) radicals with several biological HQs have been studied in previous works.[25,39] For instance, the reaction rates (k_r) of 5,7-Di-iPr-Toc• with $UQ_{10}H_2$ and α-, *β*-, and *γ*-TQH_2 in nonpolar benzene solvent are 2.33, 10.4, 10.6, and 12.2 times as large as the corresponding values in polar ethanol solvent, respectively.[25] When the logarithms of the ArO•-radical-scavenging rates (log k_s) by HQs **1**, **3**, and **5** were plotted as a function of the reciprocal of the solvent dielectric constants ($1/\epsilon$), it gave a straight line.[39] The result indicates that the reaction rates change notably, depending on the polarity of solvents,[39,40] that is, the reaction field where the antioxidants react with free radicals (Toc• and ArO•).

As listed in Table 1, the ratios of reaction rates, k_r(micelle)/k_r(2-PrOH/H_2O), of $UQ_{10}H_2$, UQ_0H_2, α-, *β*-, and *γ*-TQH_2, $TMQH_2$, and VK_3H_2 with the 7-*t*Bu-5-iPr-Toc• radical in 2-PrOH/H_2O (5:1, v/v) to those in micelle solutions are 20, 66, 28, 15, 34, 16, and 27, respectively. These antioxidants are lipophilic and thus will be localized at the inside of the micelle. Consequently, the local concentrations of HQs in a 5.0 wt % Triton X-100 micelle will become about 20 times larger than that in a homogeneous 2-propanol/water solution if we assume that the density of the part of the micelle is 1 g/mL and the volume that Triton X-100 molecules (5.0 wt %) occupy in micellar solution is 5.0% of the total volume. The 7-*t*Bu-5-iPr-Toc• radical molecule is also lipid-soluble and will react with these HQs at the inside of the micelle. Therefore, if the polarity of the reaction field is similar to that of 2-propanol/water, the k_r values of the HQs observed in the micelle will become ∼20 times larger than those in 2-propanol/water. In fact, the ratios of the reaction rates (k_r(micelle)/k_r(2-PrOH/H_2O) = 15−34) obtained for biological HQs are similar to that (=20) expected, except for the case of UQ_0H_2 (=66).

On the other hand, the reaction rate ($k_r = 3.22 \times 10^2\ M^{-1}\ s^{-1}$) of Vit C in a 5.0 wt % micelle solution is 23 times smaller than that ($k_r = 7.53 \times 10^3\ M^{-1}\ s^{-1}$) of Vit C ($Na^+AsH^-$) in 2-propanol/water, as listed in Table 1. Vit C is hydrophilic and thus will be localized at the outside of the micelle. Consequently, the concentration of Vit C in a 5.0 wt % Triton X-100 micelle will be similar to that in a homogeneous 2-propanol/water solution. The Toc• radical molecule is lipid-soluble and will react with Vit C at only the surface of the micelle. Further, the reaction field is considered to be more polar than that of 2-propanol/water. This will be the reason why the k_r value ($7.54 \times 10^3\ M^{-1}\ s^{-1}$) of $UQ_{10}H_2$ is almost the same as that ($7.53 \times 10^3\ M^{-1}\ s^{-1}$) of Vit C in 2-propanol/water, and why the k_r value ($1.48 \times 10^5\ M^{-1}\ s^{-1}$) of $UQ_{10}H_2$ is 460 times larger than that ($3.22 \times 10^2\ M^{-1}\ s^{-1}$) of Vit C in micellar solution, and why the k_r value of Vit C in a micelle is 23 times smaller than that in 2-propanol/water.

It is well-known that the polar head group of α-Toc• is localized at the surface of the biomembrane. Several kinds of phospholipids having different polar and nonpolar head groups and charges are included in the biomembrane. This may induce the change of pH on the membrane surface. The k_r values of HQs **1**−**7** are constant at broad pH region (pH < ∼9.5) (see Figure 4). The k_r values of Vit C are also constant at pHs of 6−9.[31] These results suggest that the effect of pH on the reaction rates, arising from the composition of phospholipids, is considered to be small and almost negligible in usual biomembrane systems. However, the reaction rate of Vit C will vary depending on the charge of the polar head groups of the phospholipids because Vit C takes the ascorbate monoanion (AsH^-) structure at pHs of 6−9.[33] HQs **1**−**7** exist in the neutral undissociated form without charge at pH < ∼9, as described above. The effect of the charge of the head group in phospholipids will be small.

4.3. Comparison between the Rates of the Tocopherol Regeneration Reaction with Biological Hydroquinones and Vitamin C in Animals and Plants. *(a) In human and Animals.* The reactions of hydrophilic Vit C and lipophilic $UQ_{10}H_2$ with the α-Toc• radical are well-known as usual regeneration reactions of α-Toc• in biomembrane systems, as described in the Introduction. In a previous work, the measurements of the k_r values for the reactions of the α-Toc• and 5,7-Di-iPr-Toc• radicals with biological HQs **1**−**7** were performed in 2-propanol/water (see Table 2 in ref 25). The relative rates k_r(HQ)/k_r($UQ_{10}H_2$) obtained for 7-*t*-Bu-5-iPr-Toc• ($UQ_{10}H_2$/UQ_0H_2/α-TQH_2/*β*-TQH_2/*γ*-TQH_2/$TMQH_2$/VK_3H_2 = 1.00:0.25:2.19:1.44:1.95:1.16:14.3) agreed well with those for 5,7-Di-iPr-Toc• (1.00:0.29:2.87:1.50:1.97:1.26:15.7) and α-Toc• (1.00:0.35:3.21:1.72:1.61:1.62:15.7). The result suggests that the relative reactivity of HQs **1**−**7** in homogeneous solution does not depend on the kinds of tocopheroxyl radicals used. Further, as the log k_r versus E_p plots show (Figure 5), the relative rates of k_r values (k_r(HQ)/k_r($UQ_{10}H_2$)) in 2-propanol/water and micellar solutions are similar to each other if the antioxidants are lipophilic.

TABLE 4: Concentrations of Vitamin C ([Vit C]) and Total Ubiquinone ([$UQ_{10}H_2$ + UQ_{10}]) in Several Tissues, the Values of $k_r^{Vit\ C} \times$ [Vit C] and $k_r^{UQ_{10}H_2} \times$ [$UQ_{10}H_2$ + UQ_{10}], and Their Ratios; The Values ($k_r^{Vit\ C} = 3.22 \times 10^2\ M^{-1}\ s^{-1}$ and $k_r^{UQ_{10}H_2} = 1.48 \times 10^5\ M^{-1}\ s^{-1}$) Were Used for Calculation

	[Vit C] (μM)	[$UQ_{10}H_2$ + UQ_{10}] (μM)	$k_r^{Vit\ C} \times$ [Vit C] ($M^{-1}\ s^{-1} \times \mu M$)	$k_r^{UQ_{10}H_2} \times$ [$UQ_{10}H_2$+UQ_{10}] ($M^{-1}\ s^{-1} \times \mu M$)	$k_r^{UQ_{10}H_2} \times$ [$UQ_{10}H_2$+UQ_{10}]/ $k_r^{Vit\ C} \times$ [Vit C]
human plasma	42.3 ± 8.7[a]	0.598 ± 0.176[a]	1.77×10^4	1.05×10^5	5.94
	[Vit C] (μM)	[UQ_9H_2+UQ_9] (μM)	$k_r^{Vit\ C} \times$ [Vit C] ($M^{-1}\ s^{-1} \times \mu M$)	$k_r^{UQ_{10}H_2} \times$ [UQ_9H_2+UQ_9] ($M^{-1}\ s^{-1} \times \mu M$)	$k_r^{UQ_{10}H_2} \times$ [UQ_9H_2+UQ_9]/ $k_r^{Vit\ C} \times$ [Vit C]
rat plasma	36.2 ± 1.0[b]	0.126 ± 0.0094[b]	1.17×10^4	1.86×10^4	1.59
	[Vit C] (nmol/g)	[UQ_9H_2+UQ_9] (nmol/g)	$k_r^{Vit\ C} \times$ [Vit C] ($M^{-1}\ s^{-1} \times$ nmol/g)	$k_r^{UQ_{10}H_2} \times$ [UQ_9H_2+UQ_9] ($M^{-1}\ s^{-1} \times$ nmol/g)	$k_r^{UQ_{10}H_2} \times$ [UQ_9H_2+UQ_9]/ $k_r^{Vit\ C} \times$ [Vit C]
rat prostatic	496 ± 41[b]	13.6 ± 1.2[b]	1.60×10^5	2.01×10^6	12.6
	[Vit C] (nmol/g)	[UQ_9+UQ_{10}] (nmol/g)	$k_r^{Vit\ C} \times$ [Vit C] ($M^{-1}\ s^{-1} \times$ nmol/g)	$k_r^{UQ_{10}H_2} \times$ [UQ_9+UQ_{10}] ($M^{-1}\ s^{-1} \times$ nmol/g)	$k_r^{UQ_{10}H_2} \times$ [UQ_9+UQ_{10}]/ $k_r^{Vit\ C} \times$ [Vit C]
skin in hairless mice	621[c]	15.2[c]	2.00×10^5	2.24×10^6	11.2

[a] See ref 42. [b] See ref 46. [c] See ref 47.

TABLE 5: Concentrations of Hydroquinone ([HQ]) in Envelope and Thylakoid Membranes of Spinach Leaves, the Values of $k_r^{HQ} \times$ [HQ], and Their Ratios

		envelope			thylakoid membranes		
hydroquinone	k_r^{HQ}[a] $M^{-1}\ s^{-1}$	[HQ] nmol/ mg protein	$k_r^{HQ} \times$ [HQ] $M^{-1}\ s^{-1} \times$ nmol/ mg protein	$k_r^{HQ} \times$ [HQ]/ $k_r^{PQ} \times$ [PQH_2]	[HQ] nmol/ mg protein	$k_r^{HQ} \times$ [HQ] $M^{-1}\ s^{-1} \times$ nmol/ mg protein	$k_r^{HQ} \times$ [HQ]/ $k_r^{PQ} \times$ [PQH_2]
plastoquinone-9	4.10×10^5[b]	1.60	6.56×10^5	1.00	5.21	2.14×10^6	1.00
α-tocopherol		6.50			2.55		
α-Tocopherolquinone	8.15×10^5	0.448	3.65×10^5	0.556	0.537	0.438×10^6	0.204
vitamin K_1	(1.3×10^7)[c]	0.222	(28.9×10^5)	(4.41)	0.754	(9.80×10^6)	(4.58)

[a] The values obtained in a 2-PrOH/H_2O (5:1, v/v) solution (see ref 25). [b] The k_r value of γ-TQH_2 was used for the calculation. [c] The value estimated from the reaction between vitamin K_1 hydroquinone and the 5,7-Di-iPr-tocopheroxyl radical in a 2-PrOH/H_2O (5:1, v/v) solution (see ref 25).

As listed in Table 1, the k_r values of $UQ_{10}H_2$, UQ_0H_2, α-TQH_2, β-TQH_2, γ-TQH_2, $TMQH_2$, and VK_3H_2 are 1.00, 0.25, 2.19, 1.45, 1.95, 1.17, and 14.3 times as large as that with Vit C, respectively, in 2-propanol/water, indicating that the differences in the reaction rates of HQs **1**−**7** and Vit C are not remarkable in homogeneous solution. On the other hand, the k_r values of $UQ_{10}H_2$, UQ_0H_2, α-TQH_2, β-TQH_2, γ-TQH_2, $TMQH_2$, and VK_3H_2 are 460, 385, 1430, 494, 1530, 444, and 9160 times as large as that with Vit C, respectively, in micelle solution (at pH 7). The reaction rates of HQs **1**−**7** are two to four orders of magnitude larger than that of Vit C, suggesting that these HQs may contribute significantly to the regeneration reaction of α-Toc• in biological systems.

If the HQs coexist with Vit C in tissues, the rate of regeneration of α-Toc• is represented by eq 11. The tocopherol regeneration reaction 4 of HQs may compete with reaction 2 of Vit C

$$-d[\alpha\text{-Toc}\bullet]/dt = k_r^{HQ}[HQ][\alpha\text{-Toc}\bullet] + k_r^{Vit\ C}[\text{Vit C}][\alpha\text{-Toc}\bullet] \quad (11)$$

α-TocH, Vit C, and $UQ_{10}H_2$ (and/or UQ_9H_2) are present in blood plasma, plasma lipoproteins, and all cellular membranes.[8−12,19,42−47] However, the reports that the concentrations of these antioxidants in a tissue have been determined simultaneously are very limited. The four examples are listed in Table 4. It is important to know the concentrations of reduced and oxidized ubiquinone (UQH_2 and UQ) in the tissues because the oxidized ubiquinone does not show free-radical-scavenging activity.[23−26] UQH_2 is unstable and easily oxidized to UQ during the determination of concentrations of UQH_2 and UQ. However, the rapidly extracted UQ was found to be mostly in the reduced form in both rat and human tissues.[10,48,49]

The values of $k_r^{Vit\ C} \times$ [Vit C] and $k_r^{UQ_{10}H_2} \times$ [UQ_{10} + $UQ_{10}H_2$] were calculated for Vit C and $UQ_{10}H_2$ using the concentrations listed in Table 4. If we use the k_r values obtained for 7-*t*Bu-5-iPr-Toc• in a micelle and the concentrations reported by Rijke et al.,[42] the value of the product ($k_r^{UQ_{10}H_2} \times$ [UQ_{10} + $UQ_{10}H_2$]) is 5.9 times larger than that of $k_r^{Vit\ C} \times$ [Vit C] in human plasma, suggesting that $UQ_{10}H_2$ mainly contributes to the tocopherol regeneration reactions in plasma. In fact, in human plasma or LDL undergoing oxidation, consumption of α-TocH, and formation of oxidized lipids is markedly suppressed while $UQ_{10}H_2$ is present.[20] $UQ_{10}H_2$ reduces the α-Toc• radical and is the first antioxidant consumed in LDL exposed to various oxidizing conditions. On the other hand, most α-TocH and $UQ_{10}H_2$ in plasma will be included in LDL. Each LDL contains, on average, 6−12 molecules of α-TocH per lipoprotein particle and ∼0.5−1 molecule of $UQ_{10}H_2$ per lipoprotein particle (see Table 9.1 in ref 20). Freshly and rapidly isolated LDL contains only small amounts of $UQ_{10}H_2$ when compared to that of α-TocH. If so, many LDL particles will not contain $UQ_{10}H_2$ at all. In such a case, the regeneration reaction of the α-Toc• radical will be performed by Vit C. It will be important to know the local concentration of antioxidants in order to clarify the role of each antioxidant in biological systems. On the other hand, in rat plasma, the value of the product ($k_r^{UQ_{10}H_2} \times$ [UQ_9H_2 + UQ_9]) is only 1.6 times larger than that of $k_r^{Vit\ C} \times$ [Vit C] because the concentration of total ubiquinone-9 in rat plasma is lower than that in human plasma, although the concentrations of Vit C are similar to each other.

Homma et al.[46] and Tanino et al.[47] reported the concentrations of α-TocH, Vit C, and total ubiquinone ($[UQ_9H_2 + UQ_9]$ and $[UQ_9 + UQ_{10}]$) in the prostatic of rats and in the skin of hairless mice, respectively (see Table 4). The values of the product for UQ_9H_2 are 13 and 11 times larger than that for Vit C in the prostatic of rats and the skin of hairless mice, respectively. In these tissues, we can expect that the activity of UQ_9H_2 is higher than that of Vit C.

(b) In Plants. The chloroplasts of algae and higher plants contain several prenylquinones (PQ_9, α-TQ, α-TocH, and Vit K_1).[50−52] The chloroplasto prenylquinones are bound to the photochemically active thylakoid membranes, which perform the photosynthetic electron-transport reactions. It has been reported that PQ_9, α-TQ, and Vit K_1 also exist as reduced forms in the biological systems, as well as UQ_9 and UQ_{10}.[28,53,54] The reduced forms of prenylquinone may also serve as lipid antioxidants.[25,26,39,55] Prenylquinone contents of spinach chloroplasto envelope and of isolated plastoglobuli-free spinach thylakoid membranes have been reported by Lichtenthaler et al.[50] (see Table 5). It will be interesting to compare the tocopherol regeneration activities of these HQs with that of Vit C in plants. However, as the concentrations of Vit C in the above systems have not been reported in the work of Lichtenthaler et al.,[50] we cannot compare the activities of these HQs with that of Vit C. Therefore, the comparison between the activities with the tocopherol regeneration reaction of these HQs was performed.

As described above, the relative rates of k_r values of HQs **1**−**7** in homogeneous and micellar solutions are similar to each other, being independent of the kinds of Toc•. Therefore, the relative antioxidant activities of HQs have been discussed by using the k_r values obtained for α-Toc• in 2-propanol/water (5:1, v/v) (see ref 25). As listed in Table 5, the values ($k_r^{HQ} \times [HQ]$) decrease in the order of

$$VK_1H_2 > PQ_9H_2 > \alpha\text{-}TQH_2 \qquad (12)$$

in envelope and thylakoid membranes of spinach leaves. The concentration of VK_1H_2 is the smallest, but the k_r value is 31.7 and 16.0 times larger than those of γ-TQH_2 (PQ_9H_2 model) and α-TQH_2, respectively. The result suggests that VK_1H_2 mainly contributes to the regeneration of the α-Toc• radical in envelope and thylakoid membranes of spinach leaves. However, the difference in the activity for these prenylquinones is less than 1 order of magnitude. We can expect that PQ_9H_2 also contributes to the regeneration reaction of α-Toc• in spinach leaves to some extent.

As described above, the relative rates of the regeneration reaction of α-Toc•, that is, the relative antioxidant activities of biological HQs and Vit C in several tissues of animals and plants, have been discussed using eq 11. The discussion is thought to be too simplified. However, such a discussion will be necessary to obtain the basic information with protection of oxidative damage in biological systems.

Conclusions

In the present work, we have measured the reaction rates (k_r) of the 7-*t*Bu-5-iPr-Toc• radical with seven kinds of biologically important HQs **1**−**7** and Vit C in 2-propanol/water (5:1, v/v) and 5.0 wt % Triton X-100 micellar solutions. The reaction rates (k_r) of these HQs remained constant between pHs of 6 and 9 and increased rapidly at pH ∼ 10 by increasing the pH value. It has been found that the values of k_r of $UQ_{10}H_2$, α-TQH_2, β-TQH_2, γ-TQH_2 (PQ_9H_2 model), and VK_3H_2 are 460, 1430, 494, 1530, and 9160 times as large as that of Vit C at pH 7.0 in micelle solution, respectively, although the values are similar to that of Vit C in 2-propanol/water. The relative antioxidant activities of HQs **1**−**7** and Vit C have been tentatively discussed based on the products of k_r values by their concentrations in several tissues of animals and plants. The above HQs and Vit C coexist in human, animal, and plant tissues. The result of the present kinetic study suggests that mixtures of α-TocH and these HQs may function synergistically as antioxidants in biological systems.

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