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Kinetic Study of the α-Tocopherol-Regeneration Reaction of Ubiquinol-10 in Methanol and Acetonitrile Solutions: Notable Effect of the Alkali and Alkaline Earth Metal Salts on the Reaction Rates

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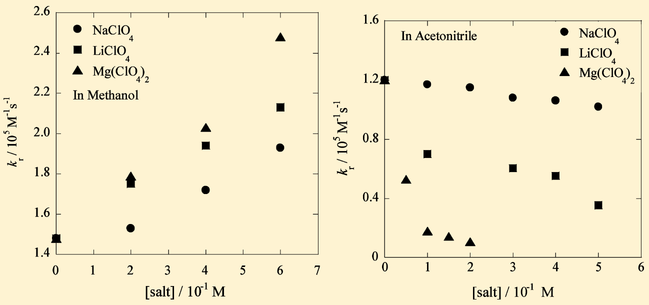

ABSTRACT: A kinetic study of regeneration reaction of α-tocopherol (α-TocH) by ubiquinol-10 has been performed in the presence of four kinds of alkali and alkaline earth metal salts ($LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$) in methanol and acetonitrile solutions, using double-mixing stopped-flow spectrophotometry. The second-order rate constants (k_r's) for the reaction of α-tocopheroxyl (α-Toc•) radical with ubiquinol-10 increased and decreased notably with increasing concentrations of metal salts in methanol and acetonitrile, respectively. The k_r values increased in the order of no metal salt < $NaClO_4$ ∼ NaI < $LiClO_4$ < $Mg(ClO_4)_2$ at the same concentration of metal salts in methanol. On the other hand, in acetonitrile, the k_r values decreased in the order of no metal salt > $NaClO_4$ ∼ NaI > $LiClO_4$ > $Mg(ClO_4)_2$ at the same concentration of metal salts. The metal salts having a smaller ionic radius of cation and a larger charge of cation gave a larger k_r value in methanol, and a smaller k_r value in acetonitrile. The effect of anion was almost negligible in both the solvents. Notable effects of metal cations on the UV−vis absorption spectrum of α-Toc• radical were observed in aprotic acetonitrile solution, suggesting complex formation between α-Toc• and metal cations. On the other hand, effects of metal cations were negligible in protic methanol, suggesting that the complex formation between α-Toc• and metal cations is hindered by the hydrogen bond between α-Toc• and methanol molecules. The difference between the reaction mechanisms in methanol and acetonitrile solutions was discussed on the basis of the results obtained. High concentrations of alkali and alkaline earth metal salts coexist with α-TocH and ubiquinol-10 in plasma, blood, and many tissues, suggesting the contribution of the metal salts to the above regeneration reaction in biological systems.

1. INTRODUCTION

It is well-known that vitamin E (α-tocopherol, α-TocH) is localized in biomembranes, plasma, and LDL and functions as an efficient inhibitor of lipid peroxidation.[1−5] 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 corresponding α-tocopheroxyl (α-Toc•) radical (reaction 1).[1,2,6] If α-TocH exists in biomembranes and edible oils, the α-Toc• radicals may react with unsaturated lipids (LH) (reaction 2). Reaction 2 is known as prooxidant reaction, which induces the degradation of unsaturated lipids.[7−11] Ubiquinol-10 ($UQ_{10}H_2$)[1,12−14] and/or vitamin C (ascorbate anion, AsH^-)[1,15−17] enhance the antioxidant activity of α-TocH by regenerating α-Toc$^•$ to α-TocH (reaction 3), to protect the above prooxidant effects. Further, α-Toc• radicals disappear by bimolecular self-reaction with another α-Toc• to give nonradical products (NRP) (reaction 4),[6,18−20] if the above ubiquinol-10 and vitamin C do not coexist in the system.

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

$$\mathrm{\alpha\text{-}Toc\bullet + LH \overset{k_p}{\rightarrow} \alpha\text{-}TocH + L\bullet} \qquad (2)$$

$$\mathrm{\alpha\text{-}Toc\bullet + UQ_{10}H_2\ (and/or\ AsH^-)} \\ \mathrm{\overset{k_r}{\rightarrow} \alpha\text{-}TocH + UQ_{10}H\bullet\ \ (and/or\ As\bullet^-)} \qquad (3)$$

$$\mathrm{\alpha\text{-}Toc\bullet + \alpha\text{-}Toc\bullet \xrightarrow{2k_d} non\text{-}radical\ products\ (NRP)} \qquad (4)$$

It is also well-known that alkali and alkaline earth metal ions (Na^+, K^+, and Mg^{2+}) are included in blood and plasma at relatively high concentrations.[21] These metal ions play important roles in many kinds of enzymatic reactions.[22] α-TocH and $UQ_{10}H_2$ coexist with these metal ions in human blood, plasma, and various tissues.[12,23,24] However, the examples of the kinetic studies of the effect of these metal ions on the free radical-scavenging reaction of the natural antioxidants, including α-TocH and $UQ_{10}H_2$, are very limited.[25−28]

In previous works,[29,30] in order to investigate the effect of alkali and alkaline earth metal ions (Li^+, Na^+, K^+, and Mg^{2+}) on the scavenging reaction of free radical by α-TocH, we measured the second-order rate constants (k_s's) for the reaction of α-TocH

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with aroxyl radical (ArO•, see Figure 1) in protic methanol and aprotic acetonitrile solutions including various metal salts (LiI, $LiClO_4$, NaI, $NaClO_4$, KI, and $Mg(ClO_4)_2$) (reaction 5), using stopped-flow spectrophotometry. The effect of these metal ions on the bimolecular self-reaction (reaction 4) was also studied.[29,30] From the results, the mechanisms for the above reactions 4 and 5 were discussed.

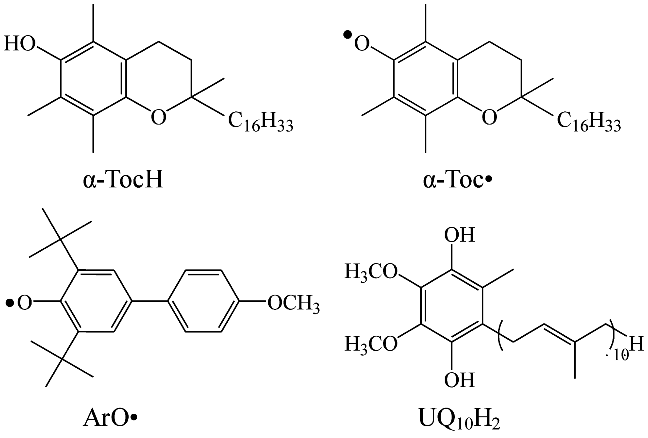

Figure 1. Molecular structures of α-tocopherol (α-TocH), α-tocopheroxyl (α-Toc•) radical, aroxyl (ArO•) radical, and ubiquinol-10 ($UQ_{10}H_2$).

$$\mathrm{ArO}\bullet + \alpha\text{-}\mathrm{TocH} \xrightarrow{k_s} \mathrm{ArOH} + \alpha\text{-}\mathrm{Toc}\bullet \qquad (5)$$

On the other hand, recently, kinetic study of the regeneration reaction of α-TocH by $UQ_{10}H_2$ (reaction 3) was carried out in ethanol (C_2H_5OH) and ethanol-d_1 (C_2H_5OD) solutions by means of double-mixing stopped-flow spectrophotometry.[14] A substantial deuterium kinetic isotope effect was observed on the second-order rate constant (k_r) and the activation energy. From the result, it was considered that proton tunneling plays an important role in the regeneration reaction of α-TocH by $UQ_{10}H_2$.

In the present work, in order to clarify the effect of alkali and alkaline earth metal ions (Li^+, Na^+, and Mg^{2+}) on the regeneration reaction of α-TocH by $UQ_{10}H_2$, we measured the rate constants (k_r's) for the reaction of α-Toc• with $UQ_{10}H_2$ in methanol and acetonitrile solutions including various metal salts ($LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$), using double-mixing stopped-flow spectrophotometry. The effect of these metal ions on the UV−vis absorption spectrum of α-Toc• was also studied.

2. EXPERIMENTAL METHODS

2.1. Materials. $LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$ are commercially available. Optical grades of methanol and acetonitrile were used for the measurement. α-Tocopherol (α-TocH) was kindly supplied from Eisai Co. Ltd. Ubiquinone-10 (UQ_{10}) was kindly supplied by Kaneka Corporation. Aroxyl radical (ArO•, 2,6-di-*tert*-butyl-4(4′-methoxyphenyl)phenoxyl) was prepared according to the method of Rieker et al.[31]

Ubiquinol-10 ($UQ_{10}H_2$) was prepared by the reduction of UQ_{10} with sodium hydrosulfite in *n*-hexane under a nitrogen atmosphere.[32,33] It was recrystallized from ethanol/petroleum ether solution: mp 46−47 °C. UV spectrum (ethanol): λ_{max} = 289 nm (ε = 4340 M^{-1} cm^{-1}) and λ_{max} = 290 nm (ε = 4010 M^{-1} cm^{-1}). The $UQ_{10}H_2$ prepared was kept under vacuum in a refrigerator at −20 °C. The purity of the $UQ_{10}H_2$ was checked spectrophotometrically before the use, using the above molar extinction coefficient (ε) in ethanol. Experiments were always performed using pure $UQ_{10}H_2$.

2.2. Measurements. Measurement of the UV−vis absorption spectrum of α-Toc• radical (see Figure 2a) was performed

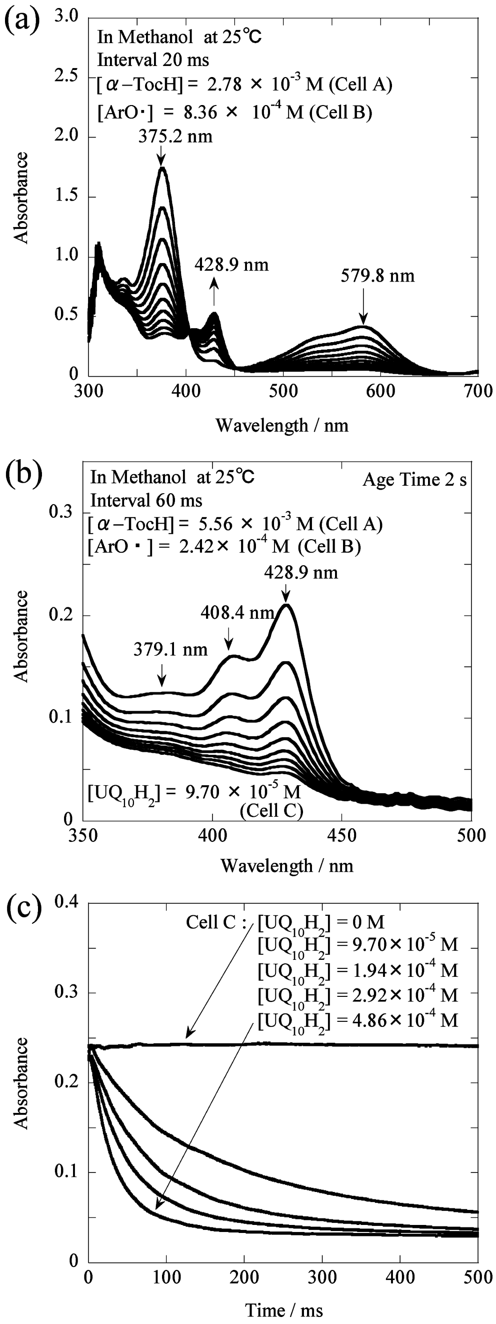

Figure 2. (a) Change in electronic absorption spectrum of ArO• and α-Toc• radicals during reaction of ArO• with α-TocH in methanol solution at 25.0 °C. The spectra were recorded at 20 ms intervals. The arrow indicates a decrease (ArO•) and an increase (α-Toc•) in absorbance with time. Initial concentrations of [α-TocH] in cell A and [ArO•] in cell B used for the reaction are shown in part a. (b) Change in absorption spectrum (at 60 ms intervals) during reaction 3 of $UQ_{10}H_2$ and α-Toc• in methanol at 25.0 °C. The initial concentration of [$UQ_{10}H_2$] in cell C was 9.70 × 10^{-5} M. The arrow indicates a decrease in absorbance of α-Toc•. (c) Absorbance decay of α-Toc• at 428.9 nm during reaction 3 in methanol at 25.0 °C.

by mixing equal volumes of methanol (or acetonitrile) solutions of ArO• and α-TocH (reaction 5) under a nitrogen atmosphere, using a Unisoku single-mixing stopped-flow spectrophotometer (model RSP-1000).

Measurement of the k_r value (reaction 3) was performed with a Unisoku double-mixing stopped-flow spectrophotometer (model RSP-1000-03F).[14] By the first mixing of equal volumes of methanol (or acetonitrile) solutions of α-TocH (cell A) and ArO• (cell B), α-Toc• radical was prepared (reaction 5), and after 2 s the second-mixing of equal volumes of α-Toc• solution and $UQ_{10}H_2$ solution (cell C) (reaction 3) was performed (see Figure 2b,c), using double-mixing unit of the RSP-1000. The rate constants (k_r's) were determined by analyzing the decay curve of α-Toc• as described later. The time between mixing two solutions and recording the first data point (that is, dead time) was 10−20 ms. The reaction was monitored with either single wavelength detection (Figure 2c) or photodiode array detector (Figure 2b) attached to the stopped-flow spectrophotometer. All the measurements were performed at 25.0 ± 0.5 °C. Experimental errors in the rate constants (k_r's) were estimated to be about 5% in methanol and acetonitrile solutions.

3. RESULTS

3.1. Effect of Metal Salts on UV−Vis Absorption Spectra of α-Tocopheroxyl Radical in Methanol and Acetonitrile Solutions. ArO• radical is stable in the absence of α-TocH, and shows absorption peaks at λ_{max} = 375.2 and 579.8 nm in methanol solution, as shown in Figure 2a.[29] By adding a methanol solution of α-TocH (2.78×10^{-3} M) to the solution of ArO• (8.36×10^{-4} M) (1:1 in volume) at 25.0 °C, the absorption peak of ArO• disappeared quickly, and the spectrum was changed to that of α-Toc• with three absorption peaks at λ_{max} = 428.9, 408.4, and 379.1 nm (reaction 5) (see Figure 2a,b), where the measurements were performed by using a single-mixing stopped-flow spectrophotometer. The absorption of ArO• radical disappeared in ~0.5 s, and changed to that of α-Toc•. α-Toc• is unstable at 25.0 °C, and its absorption decreased gradually, after passing though the maximum at ~0.5 s (data are not shown).[29]

In the present work, we studied the effect of four kinds of metal salts ($LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$) on the UV−vis absorption spectra of α-Toc• radical in methanol and acetonitrile solutions. The measurements were performed by varying the concentrations of metal salts. In methanol solution, the shift of the absorption peak of α-Toc• at λ_{max} = 428.9 nm was negligible in the presence of each metal salt used (see Figure 3), as reported in a previous work.[29] The hydrogen bond formation between α-Toc• radical and OH group of methanol may hinder the approach of the metal cations.

On the other hand, in acetonitrile solution, the shift of the absorption peak of α-Toc• was observed in the presence of metal salts.[30] The λ_{max} value (424.3 nm) of α-Toc• in the absence of metal salts increased with increasing concentrations of metal salts, and approached different constant values, depending on the kinds of metal salts, as shown in Figure 3. The λ_{max} value of α-Toc• increased in the following order at the same concentration of metal salts.

$$\text{without metal salt} < NaClO_4 \sim NaI < LiClO_4 < Mg(ClO_4)_2 \quad (6)$$

The effect of anion of the metal salts was negligible, because $NaClO_4$ and NaI show a similar shift. The result suggests the complex formation ([α-Toc•···M^+ (or M^{2+})]) between α-Toc• radical and metal cations (Li^+, Na^+, and Mg^{2+}) in acetonitrile.[30]

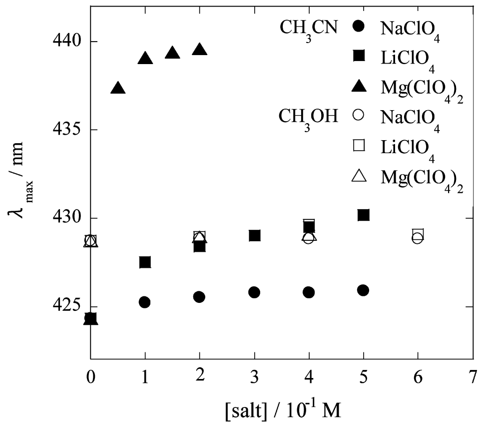

Figure 3. Plots of the peak wavelength (λ_{max}) of α-Toc• radical versus the concentration of metal salts: (a) $LiClO_4$, (b) $NaClO_4$, and (c) $Mg(ClO_4)_2$. Measurements were performed in acetonitrile and methanol solutions at 25.0 °C.

Furthermore, the effects of metal salts on the UV−vis absorption spectra of α-TocH and $UQ_{10}H_2$ included in reaction 3 were studied in methanol and acetonitrile solutions. However, any shifts of the absorption peaks of these molecules were not observed in the presence of high concentrations of metal salts (4.00×10^{-1} M) in both the solvents. The interaction between each metal cation and neutral α-TocH (or $UQ_{10}H_2$) molecule is considered to be negligible in methanol and acetonitrile. $UQ_{10}H^•$ radical may form a complex with metal cations. However, $UQ_{10}H^•$ is very unstable (lifetime τ = 1.17 μs),[34] and we were unsuccessful in observing its UV−vis spectrum in methanol and acetonitrile solutions.

3.2. Effect of Metal Salts on the Regeneration Rate (k_r) of α-Tocopherol by Ubiquinol-10 in Methanol. As described in section 2.2, measurements of the rate constant (k_r) for the reaction of α-Toc• radical with $UQ_{10}H_2$ (reaction 3) were performed in methanol solution, using a double-mixing stopped-flow spectrophotometer. For example, α-Toc• radical was prepared by the first-mixing of equal volumes of α-TocH (5.56×10^{-3} M) (cell A) and $ArO^•$ (2.42×10^{-4} M) (cell B) solutions (reaction 5), and after 2 s the second-mixing of equal volumes of α-Toc• solution and $UQ_{10}H_2$ solution (0, 9.70×10^{-5}, 1.94×10^{-4}, 2.92×10^{-4}, or 4.86×10^{-4} M) (cell C) (reaction 3) was performed (see Figure 2b). The decay curves of the absorbance of α-Toc• at 428.9 nm in methanol were shown in Figure 2c, indicating that the decay rates increase with increasing concentrations of $UQ_{10}H_2$. The decay of the absorbance of α-Toc• at 428.9 nm without $UQ_{10}H_2$ (see Figure 2c; line of $[UQ_{10}H_2]$ = 0 M) is due to a bimolecular reaction of α-Toc• radicals (reaction 4). It is slow ($2k_d = 9.2 \times 10^2$ M^{-1} s^{-1})[29] and negligible, because the reaction between α-Toc• and $UQ_{10}H_2$ is very fast ($k_r = 1.48 \times 10^5$ M^{-1} s^{-1}) (see Table 1).[14]

The reaction rate was measured by following the decrease in absorbance at 428.9 nm of the α-Toc• radical. The pseudo-first-order rate constants (k_{obsd}'s) observed at 428.9 nm were linearly dependent on the concentration of $UQ_{10}H_2$, and thus the rate law is expressed as follows

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

Table 1. Second-Order Rate Constants (k_r's) for the Reaction of Ubiquinol-10 with α-Toc• Radical in Methanol Solution Including Various Concentrations of Alkali and Alkaline Earth Metal Salts at 25.0 °C

[salt]/M	$LiClO_4$ k_r/M^{-1} s^{-1}	$NaClO_4$ k_r/M^{-1} s^{-1}	NaI k_r/M^{-1} s^{-1}	$Mg(ClO_4)_2$ k_r/M^{-1} s^{-1}
0	1.48×10^{5a}	1.48×10^{5a}	1.48×10^{5a}	1.48×10^{5a}
0.200	1.75×10^5	1.53×10^5	1.57×10^5	1.79×10^5
0.400	1.94×10^5	1.72×10^5	1.76×10^5	2.03×10^5
0.600	2.13×10^5	1.93×10^5	2.00×10^5	2.48×10^5
ionic radii of cations r/Å (six coordination)	0.76	1.02	1.02	0.72

[a] Measurement of the k_r value for [salt] = 0 M was repeated three times (1.46×10^5, 1.53×10^5, and 1.45×10^5 $M^{-1}s^{-1}$). An average value (av 1.48×10^5 M^{-1} s^{-1}) was shown in the table.

where k_r is the second-order rate constant for the reaction of α-Toc• radical with $UQ_{10}H_2$. The k_r value was obtained by plotting k_{obsd} against [$UQ_{10}H_2$], as shown in Figure 4a. The measurements of the k_r values (=1.46×10^5, 1.53×10^5, and 1.45×10^5 M^{-1} s^{-1}) were repeated three times, and the average value ($k_r^{av} = 1.48 \times 10^5$ M^{-1} s^{-1}) obtained is listed in Table 1. Experimental errors are estimated to be less than 5%.

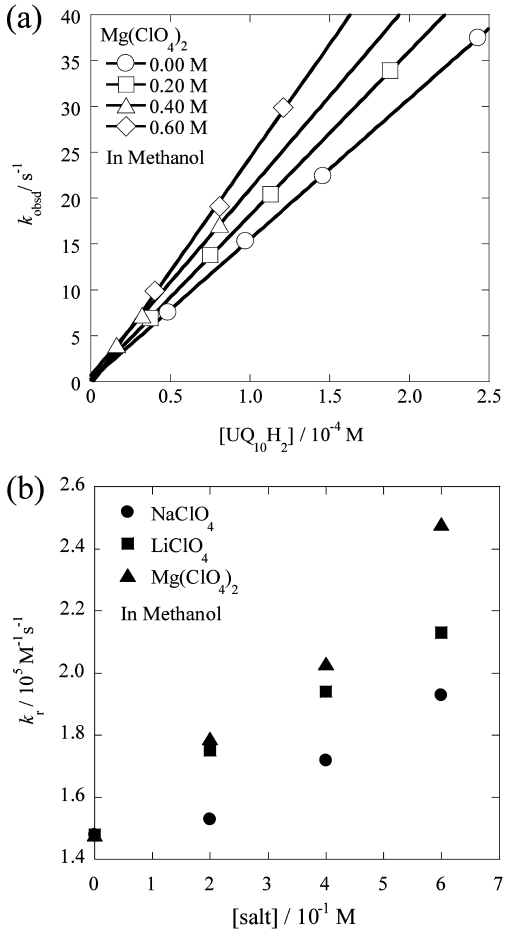

Figure 4. (a) Dependence of pseudo-first-order rate constant (k_{obsd}) on concentration of ubiquinol-10 in methanol including 0.000, 0.200, 0.400, and 0.600 M $Mg(ClO_4)_2$ salt. (b) Plots of the second-order rate constant (k_r) for the reaction of ubiquinol-10 with α-Toc• radical versus the concentration of metal salts: (a) $LiClO_4$, (b) $NaClO_4$, and (c) $Mg(ClO_4)_2$. Measurements were performed in methanol solution at 25.0 °C.

Similar measurements were performed for the reaction of α-Toc• with $UQ_{10}H_2$ in methanol solution, including various concentrations (0−6.00 × 10^{-1} M) of $LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$ salts. The results obtained for $Mg(ClO_4)_2$ salt are shown in Figure 4a. The rate constants (k_r's) obtained for $LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$ salts are listed in Table 1. The k_r values increased with increasing concentrations of metal salts, as shown in Figure 4b. Further, the k_r values increased in the following order at the same concentration of metal salts:

$$\text{without metal salt} < \mathrm{NaClO_4} \sim \mathrm{NaI} < \mathrm{LiClO_4} < \mathrm{Mg(ClO_4)_2} \tag{8}$$

The rate constants (k_r's) obtained in the presence of 6.00 × 10^{-1} M of $LiClO_4$ and $Mg(ClO_4)_2$ are 1.44 and 1.68 times larger than that in the absence of metal salts, respectively. On the other hand, the effects of $NaClO_4$ and NaI are less than those of $LiClO_4$ and $Mg(ClO_4)_2$. The reason will be discussed in the Discussion section.

3.3. Effect of Metal Salts on the Regeneration Rate (k_r) of α-Tocopherol by Ubiquinol-10 in Acetonitrile.

Similarly, measurements of the second-order rate constants (k_r's) for the reaction of α-Toc• radical with $UQ_{10}H_2$ were performed in aprotic acetonitrile solution at 25.0 °C, in the presence and absence of four kinds of metal salts ($LiClO_4$, $NaClO_4$, NaI, and $Mg(ClO_4)_2$). The rate constants (k_r's) were obtained by plotting k_{obsd} against [$UQ_{10}H_2$]. As an example, the results obtained for $Mg(ClO_4)_2$ salt are shown in Figure 5a. Notable effects of metal salts on the reaction rates (k_r) were observed, as listed in Table 2. As shown in Figure 5b, the k_r values decreased remarkably with increasing concentrations of metal salts, differing from the results obtained in methanol (see Figure 4b). Furthermore, the k_r values decreased in the following order at the same concentration of metal salts:

$$\text{without metal salt} > \mathrm{NaClO_4} \sim \mathrm{NaI} > \mathrm{LiClO_4} > \mathrm{Mg(ClO_4)_2} \tag{9}$$

The rate constants (k_r's) obtained in the presence of 5.00 × 10^{-1} M of $LiClO_4$ and $NaClO_4$ are 29% and 83% of that in the absence of metal salts, respectively. Furthermore, the rate constant (k_r) obtained in the presence of 2.00 × 10^{-1} M of $Mg(ClO_4)_2$ is 0.091% of that in the absence of metal salts. The reason for the notable decrease in the rate constants (k_r's) will be discussed in the Discussion.

4. DISCUSSION

4.1. Mechanism of the Regeneration Reaction of α-Tocopherol by Ubiquinol-10 in Methanol Solution Including Metal Salts.

Measurements of the reaction rates of galvinoxyl• (and DPPH•) radicals with α-tocopherol model without a phytyl side-chain were performed by Nakanishi et al.[25,26] in methanol solution including $Mg(ClO_4)_2$ salt. They found that the rate constants (k_s's) increase with increasing concentration of $Mg(ClO_4)_2$. Ouchi (one of the present authors) et al.[29] measured the rate constant (k_s's) for the reaction of ArO• with α-TocH (reaction 5) in methanol solutions including six kinds of metal salts (LiI, $LiClO_4$, NaI, $NaClO_4$, KI, and $Mg(ClO_4)_2$). The rate constants (k_s's) increased with increasing concentrations

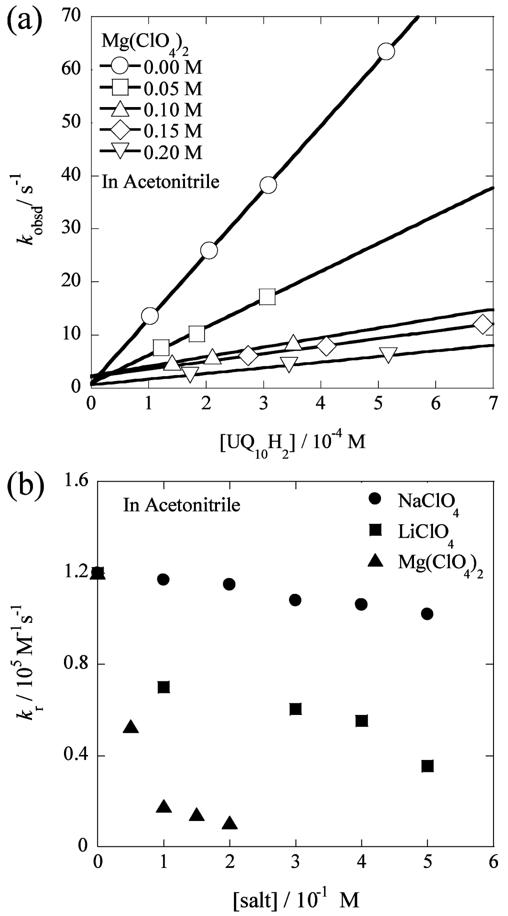

Figure 5. (a) Dependence of pseudo-first-order rate constant (k_{obsd}) on concentration of ubiquinol-10 in acetonitrile including 0.000, 0.050, 0.100, 0.150, and 0.200 M Mg(ClO$_4$)$_2$ salt. (b) Plots of the second-order rate constant (k_r) for the reaction of ubiquinol-10 with the α-Toc• radical versus the concentration of metal salts ((a) LiClO$_4$, (b) NaClO$_4$, and (c) Mg(ClO$_4$)$_2$). Measurements were performed in acetonitrile solution at 25.0 °C.

Table 2. Second-Order Rate Constants (k_r's) for the Reaction of Ubiquinol-10 with α-Toc• Radical in Acetonitrile Solution Including Various Concentrations of Alkali and Alkaline Earth Metal Salts at 25.0 °C

[metal salt]/M	LiClO$_4$ k_r/ M^{-1}s^{-1}	NaClO$_4$ k_r/ M^{-1}s^{-1}	NaI k_r/ M^{-1}s^{-1}	Mg(ClO$_4$)$_2$ k_r/ M^{-1}s^{-1}
0	1.20×10^{5a}	1.20×10^{5a}	1.20×10^{5a}	1.20×10^{5a}
0.050				5.27×10^4
0.100	7.01×10^4	1.17×10^5		1.80×10^4
0.150				1.44×10^4
0.200		1.15×10^5	1.18×10^5	1.07×10^4
0.300	6.05×10^4	1.08×10^5		
0.400	5.52×10^4	1.06×10^5	1.13×10^5	
0.500	3.55×10^4	1.02×10^5		
0.600			1.07×10^5	

[a] Measurement of the kr value for [salt] = 0 M was repeated three times (1.23×10^5, 1.21×10^5, and 1.15×10^5 M^{-1} s^{-1}). An average value (av 1.20×10^5 M^{-1} s^{-1}) was shown in the table.

of metal salts. Furthermore, the k_s values increased in the following order at the same concentration of metal salts:

$$\text{without metal salt} < \text{KI} \sim \text{NaClO}_4 \sim \text{NaI} \leq \text{LiClO}_4 < \text{Mg(ClO}_4)_2 < \text{LiI} \qquad (10)$$

From the results, it has been suggested that reaction 5 proceeds via an electron-transfer intermediate [ArO:$^-$···α-TocH•$^+$] in methanol (see Figure 7 in ref 29).

In the present work, measurement of the rate constant (k_r) for the reaction of α-Toc• with UQ$_{10}$H$_2$ was also performed in protic methanol solution including LiClO$_4$, NaClO$_4$, NaI, and Mg(ClO$_4$)$_2$ salts. As described in the Results section, the changes of UV−vis absorption spectra of α-Toc•, α-TocH, and UQ$_{10}$H$_2$ were negligible in methanol in the presence of four metal salts (4.00×10^{-1} M), showing that the interactions between these neutral molecules and the cations (and anions) of metal salts are negligibly weak. Similarly, the interaction between UQ$_{10}$H• and the cations (and anions) of metal salts is thought to be negligible in methanol, although we could not observe the absorption spectrum of UQ$_{10}$H• radical because of its instability.[34] The results suggest that both the total energy levels of the reactants (α-Toc• + UQ$_{10}$H$_2$) and the reaction products (α-TocH + UQ$_{10}$H•) in reaction 3 do not vary in the presence of metal salts, as shown in Figure 6a.

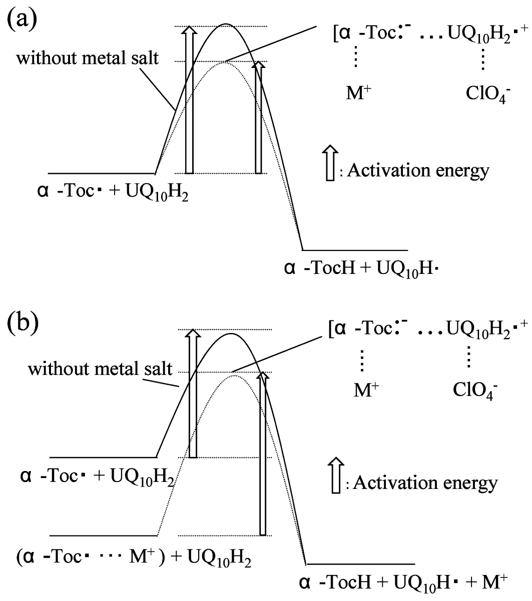

Figure 6. (a) Mechanism of the α-tocopherol-regeneration reaction of UQ$_{10}$H$_2$ in methanol. The figure shows that the transition state for the reaction of α-Toc• with UQ$_{10}$H$_2$ has the property of an electron-transfer intermediate [α-Toc:$^-$···UQ$_{10}$H$_2$•$^+$]. The effects of metal salts on the reaction rates (k_r's) may be explained by considering two kinds of equilibrium between (i) α-Toc:$^-$ anion and M$^+$ cation and (ii) UQ$_{10}$H$_2$•$^+$ cation and ClO$_4^-$ anion at the transition state (see text). (b) Mechanism of the α-tocopherol-regeneration reaction of UQ$_{10}$H$_2$ in acetonitrile. The energy level of the reactants (α-Toc• + UQ$_{10}$H$_2$) is lowered by the complex formation between α-Toc• and metal cations (M$^+$ (or M^{2+})) (see text).

As shown in Figure 4b, the k_r values increased in the order of eq 8 in methanol. The order of eq 8 is the same as that of eq 10, suggesting that reaction 3 in methanol also proceeds via an electron-transfer intermediate. As shown in Figure 6a, if the transition state of the α-Toc•-scavenging reaction by UQ$_{10}$H$_2$ has the property of an electron-transfer intermediate [α-Toc:$^-$···UQ$_{10}$H$_2$•$^+$], the metal cations (Li$^+$, Na$^+$, and Mg^{2+}) will interact with α-Toc:$^-$ anion by Coulomb interaction and form a complex with a α-Toc:$^-$ anion [α-Toc:$^-$···M$^+$ (or M^{2+})].[29] Similarly, the ClO$_4^-$ anion of the salts will also interact with UQ$_{10}$H$_2$•$^+$ cation and form a complex with an UQ$_{10}$H$_2$•$^+$ cation

$[\text{UQ}_{10}\text{H}_2\bullet^+\cdots\text{ClO}_4^-]$. As a result, the energy level of an electron-transfer intermediate $[\alpha\text{-Toc:}^-\cdots\text{UQ}_{10}\text{H}_2\bullet^+]$ will be stabilized (that is, the activation energy (E_{act}) will decrease), and thus, the k_r values increase. The Coulomb interaction energy will increase with decreasing the ionic radius of cations (Na^+, Li^+, and Mg^{2+}) and with increasing the cation charge from +1e to +2e (see Table 1). In fact, the rate constants (k_r's) increase in the order of eq 8. The k_r value observed for NaI showed a little bit larger value than that for NaClO_4, although the difference is very small. As the ionic radii of anions (ClO_4^- and I^-) are larger than those of cations, the effect of the anion may be smaller than that of the cation. The details of the effect of the anion are not clear at present.

4.2. Mechanism of the Regeneration Reaction of α-Tocopherol by Ubiquinol-10 in Acetonitrile Solution Including Metal Salts.

In the present work, measurement of the rate constant (k_r) for the reaction of α-Toc• with UQ_{10}H_2 was performed in aprotic acetonitrile solution including LiClO_4, NaClO_4, NaI, and $\text{Mg(ClO}_4)_2$ salts. As shown in Figure 5 and listed in Table 2, a notable effect of metal salts on the rate constants (k_r) was observed in acetonitrile. The rate constants (k_r's) decreased rapidly with increasing concentrations of metal salts. Furthermore, the k_r values decreased in the order of eq 9 at the same concentration of metal salts, differing from the increase of the k_r values in methanol.

As described in section 3.1, a notable effect of the metal salts (LiClO_4, NaClO_4, NaI, and $\text{Mg(ClO}_4)_2$) on the UV−vis absorption spectrum of α-Toc• was observed in acetonitrile. As shown in Figure 3, the $\lambda_{\max}$ value (424.3 nm) of α-Toc• without metal salts increased with increasing concentrations of metal salts in acetonitrile, suggesting the $[\alpha\text{-Toc}\bullet\cdots\text{M}^+$ (or M^{2+})] complex formation between α-Toc• radical and metal cations.[25,30] The effect of anion of the metal salts is negligible, because NaClO_4 and NaI show a similar shift (data are not shown). If M^+ ion forms a (1:1) complex with α-Toc•, the following equilibrium between α-Toc• and M^+ ion may exist in acetonitrile solution.

$$\alpha\text{-Toc}\bullet + \text{M}^+ \overset{K}{\rightleftharpoons} [\alpha\text{-Toc}\bullet\cdots\text{M}^+] \qquad (11)$$

Here, K is a stability constant for eq 11. With increasing concentrations of the metal salts, the concentrations of the complex ($[\alpha\text{-Toc}\bullet\cdots\text{M}^+]$) will increase gradually, and thus, the shifts of the absorption maximum ($\lambda_{\max}$) proceed. Recently, the stability constant (K) was determined to be 9.2, 2.8, and 45 M^{-1} for LiClO_4, NaClO_4, and $\text{Mg(ClO}_4)_2$, respectively.[30] The value increases in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg(ClO}_4)_2$. The result indicates that the metal cation having smaller ionic radius and larger positive charge of +2e gives a larger shift of the $\lambda_{\max}$ value and a larger K value.

As described above, a notable red shift of the $\lambda_{\max}$ value of the α-Toc• was observed in the presence of the metal salts in acetonitrile, indicating the complex formation between α-Toc• and metal cations. On the other hand, the shift of the $\lambda_{\max}$ value of UQ_{10}H_2 (and α-TocH) was negligible in acetonitrile in the presence of metal salts, showing that the interactions between UQ_{10}H_2 (and α-TocH) and metal cations are negligibly weak. The results suggest that the total energy level of the reactants (α-Toc• + UQ_{10}H_2) in reaction 3 will be lowered by the complex formation (see Figure 6b). In such a case, the decrease in the reaction rate constant (k_r) will be observed in the presence of metal salts, as expected from the Evans−Polanyi theory.[35,36] Further, the difference between the rate constants (k_r's) for NaClO_4, LiClO_4, and $\text{Mg(ClO}_4)_2$ will be explained in the following way: as the stability constant (K) increases in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg(ClO}_4)_2$, the stabilization energy of the reactants (α-Toc• + UQ_{10}H_2) will increase in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg(ClO}_4)_2$. Consequently, the activation energy for reaction 3 increases in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg(ClO}_4)_2$, resulting in the decrease in the k_r value in the order of $\text{NaClO}_4 > \text{LiClO}_4 > \text{Mg(ClO}_4)_2$ (see Figure 6b).

As reaction 3 indicates, the products for reaction 3 are α-TocH + $\text{UQ}_{10}\text{H}\bullet$. We may expect the complex formation $[\text{UQ}_{10}\text{H}\bullet\cdots\text{M}^+$ (or M^{2+})] between $\text{UQ}_{10}\text{H}\bullet$ radical and metal cations in acetonitrile solution. However, we could not observe the UV−vis absorption spectrum of $\text{UQ}_{10}\text{H}\bullet$ radical, because the $\text{UQ}_{10}\text{H}\bullet$ radical is unstable (half-life τ = 1.17 μs)[34] and immediately changes to UQ_{10} and UQ_{10}H_2 by disproportionation reaction. Therefore, we could not ascertain a lowering of the total energy level of the reaction products (α-TocH + $\text{UQ}_{10}\text{H}^\bullet$). The result of the present kinetic study indicates that a lowering of the energy level of the α-Toc• due to the complex formation mainly contributes to the decrease of the reaction rates (k_r's) in acetonitrile.

4.3. Alkali and Alkaline Earth Metal Salts May Affect the Regeneration Reaction of α-Tocopherol by Ubiquinol-10 in Biological Systems and the Prooxidant Reaction of α-Tocopherol in Edible Oils.

As described in the Introduction, α-TocH, UQ_{10}H_2, and alkali and alkaline earth metal ions (Na^+, K^+, Mg^{2+}, and Ca^{2+}) coexist in blood, plasma, plasma lipoproteins, and cellular membranes.[21−24] For instance, high concentrations of Na^+ and K^+ ions are included in human blood ($[\text{Na}^+]$ = 81.7 mM and $[\text{K}^+]$ = 44.6 mM) and plasma ($[\text{Na}^+]$ = 136 mM and $[\text{K}^+]$ = 3.6 mM).[21] The concentrations of metal cations found in mammals are $[\text{Na}^+]$ = 140 mM, $[\text{K}^+]$ = 4 mM, $[\text{Mg}^{2+}]$ = 1.5 mM in extracellular fluid and $[\text{Na}^+]$ = 10 mM, $[\text{K}^+]$ = 140 mM, $[\text{Mg}^{2+}]$ = 30 mM in intracellular fluids.[37] The concentrations of α-TocH and UQ_{10}H_2 in human plasma were reported to be av 22.0 (12.0−36.0) μM[23,24] and av 0.71 (0.36−1.10) μM,[12] respectively. Therefore, the concentrations of Na^+, K^+, and Mg^{2+} ions are much higher than those of α-TocH and UQ_{10}H_2 in human plasma.

Na^+ ion has the highest concentration ($[\text{Na}^+]$ = 136 mM) among alkali and alkaline earth metal ions in plasma. As listed in Tables 1 and 2, the k_r values in methanol and acetonitrile with 136 mM of Na^+ ion are by ~3% larger and by ~3% smaller than those without Na^+ ion, respectively. Similarly, the k_r values in methanol and acetonitrile with 30 mM of Mg^{2+} ion are by ~3% larger and by ~34% smaller than those without Mg^{2+} ion, respectively. The effects of Na^+ ion on the α-tocopherol-regenerating rate constants (k_r's) are small and negligible in methanol and acetonitrile solutions. On the other hand, the effect of Mg^{2+} ion on the reaction rate (k_r) is notable in acetonitrile.

As described in the Introduction, α-Toc• radical is an important key radical, which appears in the process of the antioxidant and prooxidant actions of α-TocH (see reactions 1−4). In the present work, a detailed kinetic study has been performed for regeneration reaction 3, and the mechanisms involved and the effect of metal salts on the reaction rates (k_r) in protic methanol and aprotic acetonitrile solutions have been studied.

As described in section 4.2, α-Toc• radical molecule forms a 1:1 complex with metal cations in acetonitrile. When α-Toc• forms a complex with metal cations (such as Na^+, K^+, and Mg^{2+} ions), the total energy levels of the reactants (α-Toc• + LH) in reaction 2 will decrease,[30] and thus, the k_p value will decrease.[11] That is, the prooxidant effect of α-TocH, which is observed in aprotic edible oils including high concentration of α-TocH, may be suppressed by the addition of the metal salts (NaCl, KCl, $MgCl_2$, and $CaCl_2$). We can expect notable effects of metal salts on the above biologically important reactions 2 and 3. However, the rate constant (k_r) increased and decreased with increasing concentrations of metal salts, depending on the kinds of the solvents used for the reaction (that is, the character of the reaction field). It will be interesting to investigate the effects of metal salts on the reaction rates (k_p and k_r) in model membrane systems, such as micellar solution or liposome.

5. SUMMARY

A kinetic study of regeneration reaction of α-TocH by $UQ_{10}H_2$ has been performed in the presence of four kinds of alkali and alkaline earth metal salts in methanol and acetonitrile solutions, using double-mixing stopped-flow spectrophotometry. In protic methanol solution, the second-order rate constant (k_r) for the reaction of α-Toc• radical with $UQ_{10}H_2$ increased, with increasing concentrations of metal salts, and it increased in the following order at the same concentration of metal salts: no metal salt $<$ $NaClO_4$ $\sim$ NaI $<$ $LiClO_4$ $<$ $Mg(ClO_4)_2$. On the other hand, in aprotic acetonitrile solution, the rate constant (k_r) decreased notably, with increasing concentrations of metal salts, and it decreased in the following order at the same concentration of metal salts: no metal salt $>$ $NaClO_4$ $\sim$ NaI $>$ $LiClO_4$ $>$ $Mg(ClO_4)_2$. The decrease of the k_r value in acetonitrile will be due to the complex formation between α-Toc• and metal cations. The alkali and alkaline earth metal salts having smaller ionic radius of cation and larger cation charge gave a larger k_r value in methanol solution, and a smaller k_r value in acetonitrile solution. From the results, the mechanisms for the above two different types of reactions observed in methanol and acetonitrile solutions were discussed.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Niki, E. *Chem. Phys. Lipids* **1987**, *44*, 227−253.

(2) Barclay, L. R. C. *Can. J. Chem.* **1993**, *71*, 1−16.

(3) Traber, M. G.; Atkinson, J. *Free Radical Biol. Med.* **2007**, *43*, 4−15.

(4) Esterbauer, H.; Dieber-Rotheneder, M.; Striegel, G.; Waeg, G. *Am. J. Clin. Nutr.* **1991**, *53*, 314S−321S.

(5) Neuzil, J.; Thomas, S. R.; Stocker, R. *Free Radical Biol. Med.* **1997**, *22*, 57−71.

(6) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053−7065.

(7) Cillard, J.; Cillard, P.; Cormier, M.; Girre, L. *J. Am. Oil Chem. Soc.* **1980**, *57*, 252−255.

(8) Terao, J.; Matsushita, S. *Lipids* **1986**, *21*, 255−260.

(9) Bowry, V. W.; Stocker, R. *J. Am. Chem. Soc.* **1993**, *115*, 6029−6044.

(10) Mukai, K.; Noborio, S.; Nagaoka, S. *Int. J. Chem. Kinet.* **2005**, *37*, 605−610.

(11) Ouchi, A.; Ishikura, M.; Konishi, K.; Nagaoka, S.; Mukai, K. *Lipids* **2009**, *44*, 935−943.

(12) *Coenzyme Q: Molecular Mechanisms in Health and Disease*; Kagan, V. E., Quinn, P. J., Eds.; CRC Press: Boca Raton, FL, 2001.

(13) Mukai, K.; Itoh, S.; Morimoto, H. *J. Biol. Chem.* **1992**, *267*, 22277−22281.

(14) Ouchi, A.; Nagaoka, S.; Mukai, K. *J. Phys. Chem. B* **2010**, *114*, 6601−6607.

(15) Packer, J. E.; Slater, T. F.; Willson, R. L. *Nature* **1979**, *278*, 737−738.

(16) Bisby, R. H.; Parker, A. W. *Arch. Biochem. Biophys.* **1995**, *317*, 170−178.

(17) Nagaoka, S.; Kakiuchi, T.; Ohara, K.; Mukai, K. *Chem. Phys. Lipids* **2007**, *146*, 26−32.

(18) Lucarini, M.; Pedulli, G. F.; Cipollone, M. *J. Org. Chem.* **1994**, *59*, 5063−5070.

(19) Gregor, W.; Grabner, G.; Adelwhrer, C.; Rosenau, T.; Gille, L. *J. Org. Chem.* **2005**, *70*, 3472−3483.

(20) Mukai, K.; Ouchi, A.; Mitarai, A.; Ohara, K.; Matsuoka, C. *Bull. Chem. Soc. Jpn.* **2009**, *82*, 494−503.

(21) Takeda, R.; Nakamura, T.; Saito, Y.; Takeda, A.; Yamashita, C.; Shigetomi, H.; Takeda, T.; Kimura, M. *Biomed. Res. Trace Elem.* **2007**, *18*, 277−280.

(22) Stryer, L. *Biochemistry*, 3rd ed.; W. H. Freeman and Company: New York, 1988.

(23) Colome, C.; Artuch, R.; Vilaseca, M.-A.; Sierra, C.; Brandi, N.; Lambruschini, N.; Cambra, F. J.; Campistol, J. *Am. J. Clin. Nutr.* **2003**, *77*, 185−188.

(24) Machlin, L. J. Vitamin E. In *Handbook of Vitamins*; Machlin, L. J., Ed.; Marcel Dekker Inc.: New York, 1991; pp 99−144.

(25) Nakanishi, I.; Fukuhara, K.; Shimada, T.; Ohkubo, K.; Iizuka, Y.; Inami, K.; Mochizuki, M.; Urano, S.; Itoh, S.; Miyata, N.; Fukuzumi, S. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1520−1524.

(26) Nakanishi, I.; Kawashima, T.; Ohkubo, K.; Kanazawa, H.; Inami, K.; Mochizuki, M.; Fukuhara, K.; Okuda, H.; Ozawa, T.; Itoh, S.; Fukuzumi, S.; Ikota, N. *Org. Biomol. Chem.* **2005**, *3*, 626−629.

(27) Nakanishi, I.; Miyazaki, K.; Shimada, T.; Ohkubo, K.; Urano, S.; Ikota, N.; Ozawa, T.; Fukuzumi, S.; Fukuhara, K. *J. Phys. Chem. A* **2002**, *106*, 11123−11126.

(28) Nakanishi, I.; Shimada, T.; Ohkubo, K.; Manda, S.; Shimizu, T.; Urano, S.; Okuda, H.; Miyata, N.; Ozawa, T.; Anzai, K.; Fukuzumi, S.; Ikota, N.; Fukuhara, K. *Chem. Lett.* **2007**, *36*, 1276−1277.

(29) Ouchi, A.; Nagaoka, S.; Abe, K.; Mukai, K. *J. Phys. Chem. B* **2009**, *113*, 13322−13331.

(30) Kohno, Y.; Fujii, M.; Matsuoka, C.; Hashimoto, H.; Ouchi, A.; Nagaoka, S.; Mukai, K. *J. Phys. Chem. B* **2011**, *115*, 9880−9888.

(31) Rieker, A.; Scheffler, K. *Liebigs Ann. Chem.* **1965**, *689*, 78−92.

(32) Podda, M.; Weber, C.; Traber, M. G.; Packer, L. *J. Lipid Res.* **1996**, *37*, 893−901.

(33) Mukai, K.; Tokunaga, A.; Itoh, S.; Kanesaki, Y.; Ohara, K.; Nagaoka, S.; Abe, K. *J. Phys. Chem. B* **2007**, *111*, 652−662.

(34) Parker, A. W.; Hester, R. E.; Phillips, D.; Umapathy, S. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2649−2653.

(35) Evans, M. G.; Polanyi, M. *Trans. Faraday Soc.* **1938**, *34*, 11−24.

(36) Kagiya, T.; Sumida, Y.; Inoue, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 2422−2425.

(37) Murray, R. K.; Granner, D. K.; Mayes, P. A.; Rodwell, V. W. Membranes: Structure, Assembly, and Function. *Harper's Biochemistry*, 25th ed.; McGraw-Hill: New York, 2000; Chapter 43, pp 505−533.