

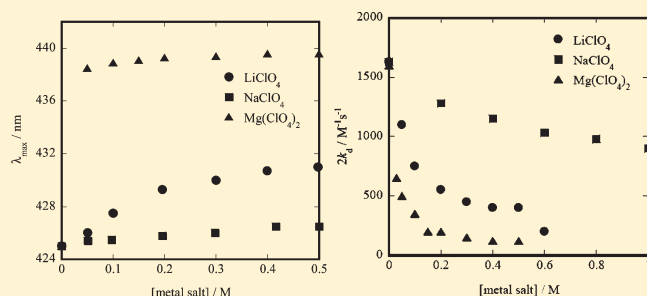
Notable Effects of the Metal Salts on the Formation and Decay Reactions of α -Tocopheroxyl Radical in Acetonitrile Solution. The Complex Formation between α -Tocopheroxyl and Metal Cations

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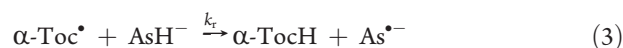
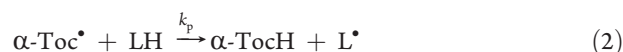
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ABSTRACT: The measurement of the UV–vis absorption spectrum of α -tocopheroxyl (α -Toc $^{\bullet}$) radical was performed by reacting aroxyl (ArO^{\bullet}) radical with α -tocopherol (α -TocH) in acetonitrile solution including four kinds of alkali and alkaline earth metal salts (MX or MX_2) (LiClO_4 , LiI , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$), using stopped-flow spectrophotometry. The maximum wavelength (λ_{max}) of the absorption spectrum of the α -Toc $^{\bullet}$ at 425.0 nm increased with increasing concentration of metal salts (0–0.500 M) in acetonitrile, and it approached constant values, suggesting an $[\alpha\text{-Toc}^{\bullet}\text{-M}^+ \text{ (or } \text{M}^{2+})]$ complex formation. The stability constants (K) were determined to be 9.2, 2.8, and 45 M^{-1} for LiClO_4 , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$, respectively. By reacting ArO^{\bullet} with α -TocH in acetonitrile, the absorption of ArO^{\bullet} disappeared rapidly, while that of α -Toc $^{\bullet}$ appeared and then decreased gradually as a result of the bimolecular self-reaction of α -Toc $^{\bullet}$ after passing through the maximum. The second-order rate constants (k_s) obtained for the reaction of α -TocH with ArO^{\bullet} increased linearly with an increasing concentration of metal salts. The results indicate that the hydrogen transfer reaction of α -TocH proceeds via an electron transfer intermediate from α -TocH to ArO^{\bullet} radicals followed by proton transfer. Both the coordination of metal cations to the one-electron reduced anions of ArO^{\bullet} ($\text{ArO}^{\bullet-}$) and the coordination of counteranions to the one-electron oxidized cations of α -TocH ($\alpha\text{-TocH}^{+\bullet}$) may stabilize the intermediate, resulting in the acceleration of electron transfer. A remarkable effect of metal salts on the rate of bimolecular self-reaction ($2k_d$) of the α -Toc $^{\bullet}$ radical was also observed. The rate constant ($2k_d$) decreased rapidly with increasing concentrations of the metal salts. The $2k_d$ value decreased at the same concentration of the metal salts in the following order: no metal salt > NaClO_4 > LiClO_4 > $\text{Mg}(\text{ClO}_4)_2$. The complex formation between α -Toc $^{\bullet}$ and metal cations may stabilize the energy level of the reactants ($\alpha\text{-Toc}^{\bullet} + \alpha\text{-Toc}^{\bullet}$), resulting in the decrease of the rate constant ($2k_d$). The alkali and alkaline earth metal salts having a smaller ionic radius of cation and a larger charge of cation gave larger K and k_s values and a smaller $2k_d$ value.



1. INTRODUCTION

Vitamin E (α -tocopherol, α -TocH) is well-known as a representative lipophilic antioxidant.^{1–3} The antioxidant actions of α -tocopherols have been ascribed to the scavenging reaction of peroxy (LOO^{\bullet}) radicals, producing the corresponding α -tocopheroxyl (α -Toc $^{\bullet}$, Figure 1) radicals (reaction 1).⁴ If α -tocopherols exist in biomembranes and oils, the α -Toc $^{\bullet}$ radicals may react with unsaturated lipids (LH) (reaction 2). Reaction 2 is known as a prooxidant reaction, which induces the degradation of unsaturated lipids.^{5–9} α -Toc $^{\bullet}$ radicals may be reduced to α -TocH by vitamin C (ascorbate anion, AsH^-) (reaction 3)^{1,10–12} and/or ubiquinol-10 (UQ_{10}H_2)^{13–15} in biomembranes, to protect the above prooxidant effects. Further, α -Toc $^{\bullet}$ radicals disappear by bimolecular reaction with another α -Toc $^{\bullet}$ to give nonradical products (NRP) (reaction 4).⁴



As described above, α -Toc $^{\bullet}$ radical is an important key radical, which appears in the process of the antioxidant and prooxidant actions of α -TocH. Detailed kinetic studies have been performed for reactions 1,^{2,4} 2,⁹ 3,^{10–15} and 4,^{4,16–18} and the mechanisms involved have been studied extensively.

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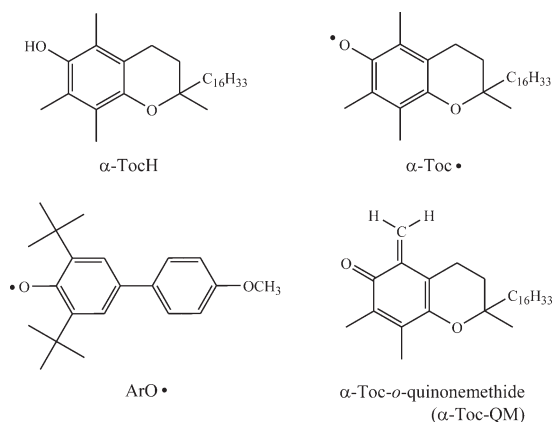


Figure 1. Molecular structures of α -tocopherol (α -TocH), α -tocopheroxyl (α -Toc \cdot) radical, aroxyl (ArO \cdot) radical, and α -Toc-*o*-quinonemethide (α -Toc-QM).

It is also well-known that relatively high concentrations of alkali and alkaline earth metal ions (Na^+ , K^+ , Mg^{2+} , and Ca^{2+}) are included in human blood, plasma, and various tissues.¹⁹ These metal ions play important roles for many kinds of enzymatic reactions.²⁰ α -TocH coexists with these metal ions in the above biological systems.^{21,22} However, the examples of the kinetic studies of the effect of these metal ions on the free radical-scavenging reaction of the antioxidants, including α -TocH, are very limited.^{23–26}

Nakanishi et al.^{23,24} measured the reaction rates of galvinoxyl \cdot and DPPH \cdot (1,1-diphenyl-2-picrylhydrazyl) radicals with the α -TocH model without a phytyl side-chain in methanol in the presence of $\text{Mg}(\text{ClO}_4)_2$ (0–0.4 M) and found that the second-order rate constants (k_s) increase, with an increasing concentration of $\text{Mg}(\text{ClO}_4)_2$. On the other hand, no effect of $\text{Mg}(\text{ClO}_4)_2$ on the rate constant (k_d) was observed in acetonitrile. In a previous work, to clarify the effect of alkali and alkaline earth metal ions on the scavenging reactions of free radicals by α -TocH, we measured the rate constant (k_s) for the reaction of α -TocH with 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxy (aroxyl, ArO \cdot) (see Figure 1) in protic methanol solvent including various concentrations of metal salts (LiI, LiClO_4 , NaI, NaClO_4 , KI, and $\text{Mg}(\text{ClO}_4)_2$) (reaction 5), using stopped-flow spectrophotometry.²⁷ The effect of these metal ions on the bimolecular self-reaction rate ($2k_d$) of α -Toc \cdot (reaction 4) was also studied.



In the present work, kinetic studies were performed for reactions 4 and 5 in aprotic acetonitrile solvent including various concentrations of metal salts (LiClO_4 , LiI, NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$), to clarify the effect of the reaction-field (that is, media effect) on the above rate constants (k_s and $2k_d$). Some notable effects of the metal salts were observed for (i) the rate constant (k_s) (reaction 5), (ii) the rate constant ($2k_d$) of bimolecular self-reaction of α -Toc \cdot (reaction 4), and (iii) the maximum wavelength (λ_{max}) (and the molar extinction coefficient (ϵ_{max})) of α -Toc \cdot in acetonitrile, although the effects of metal salts were not observed for the $2k_d$ and λ_{max} (and ϵ_{max}) values in methanol solution.²⁷ From the results, the mechanisms for reactions 4 and 5 were discussed.

2. EXPERIMENTAL METHODS

2.1. Materials. α -Tocopherol was kindly supplied from Eisai Co. Ltd. LiClO_4 , LiI, NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$ are commercially

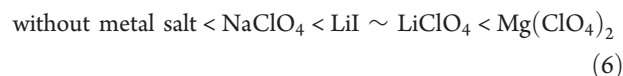
available. Optical grade of acetonitrile was used for the measurement. Aroxyl (ArO \cdot) radical was prepared according to the method of Rieker et al.²⁸

2.2. Measurements. The kinetic data were obtained with a Unisoku model RSP-1000 stopped-flow spectrophotometer by mixing equal volumes of solutions of antioxidants and ArO \cdot under nitrogen atmosphere. The shortest time necessary for 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 or a photodiode array detector attached to the stopped-flow spectrophotometer. All measurements were performed at 25.0 ± 0.5 °C. Experimental errors in the rate constants (k_s and $2k_d$) were estimated to be less than 5 and 7%, respectively, in acetonitrile solution.

3. RESULTS

3.1. Effect of Metal Salts on UV–Vis Absorption Spectrum of α -Tocopheroxyl Radical in Acetonitrile Solution. The ArO \cdot radical is stable in the absence of α -TocH and shows absorption peaks at $\lambda_{\text{max}} = 375.0$ nm ($\epsilon = 19\,100$ M $^{-1}$ cm $^{-1}$) and 580.0 nm ($\epsilon = 4200$ M $^{-1}$ cm $^{-1}$) in acetonitrile solution, as shown in Figure 2a. By adding the acetonitrile solution of α -TocH (4.80×10^{-3} M) to the solution of ArO \cdot (1.25×10^{-4} M) (1:1 in volume) at 25.0 °C, the absorption peak of ArO \cdot disappeared quickly, and the spectrum was changed to that of α -Toc \cdot with two absorption peaks at $\lambda_{\text{max}} = 425.0$ and 405.1 nm (reaction 5) (see Figure 2a).

Similar measurements were performed in acetonitrile solution, including various concentrations of LiClO_4 , LiI, NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$ (MX (or MX_2)) salts, where the same concentration of ArO \cdot was used for each measurement. As shown in parts a–c of Figure 3, shifts of the absorption peak (λ_{max}) of α -Toc \cdot were observed in the presence of the metal salts LiClO_4 , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$, respectively. The λ_{max} value (425.0 nm) of α -Toc \cdot in the absence of metal salts increased, with increasing concentrations of metal salts, and approached different constant values, depending on the kind of metal salt, as shown in Figure 4 and as listed in Table 1. The λ_{max} values were similar to each other in the solution including LiI and LiClO_4 salts, indicating that the effect of anion is negligible. The λ_{max} value increased in the following order at the same concentration of the metal salts.



The result suggests the complex formation ($[\alpha\text{-Toc}\cdot\text{M}^+ \text{ (or } \text{M}^{2+})]$) between α -Toc \cdot radical molecule and metal cations ($\text{M}^+ = \text{Li}^+$ or Na^+ , and $\text{M}^{2+} = \text{Mg}^{2+}$) in acetonitrile. On the other hand, such a complex formation was not observed in methanol solution, as reported in a previous work.²⁷

Furthermore, we studied the effect of the metal salts on the UV–vis absorption spectra of ArO \cdot ($\lambda_{\text{max}} = 375.0$ and 580.0 nm), α -TocH (294.0 nm), and ArOH (266.0 nm) molecules included in reaction 5 in acetonitrile. However, the shifts of the absorption peaks of these molecules were not observed in the presence of every metal salt (0.400 M) used. The interactions between metal cations (and anions) and these molecules are considered to be negligible in acetonitrile.

In addition to the shift of the absorption peak (λ_{max}), an increase of the absorbance (that is, an increase of the molar extinction coefficient (ϵ)) of α -Toc \cdot radical was observed, with

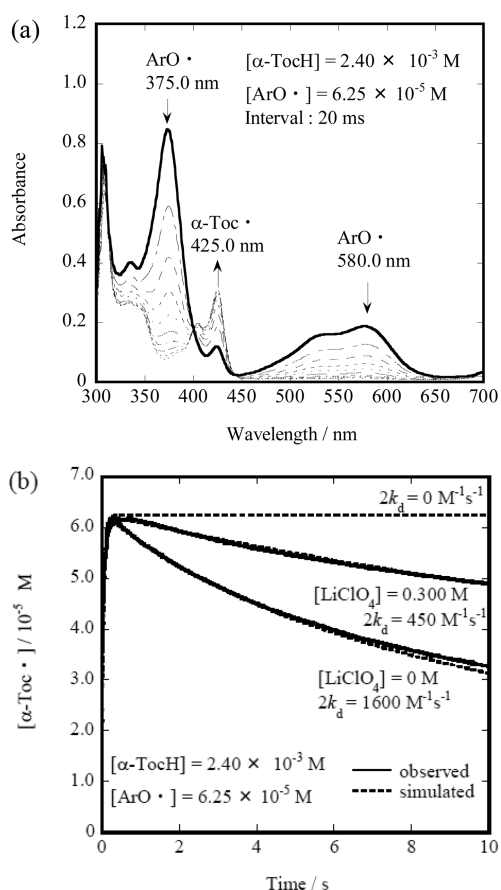


Figure 2. (a) Change in electronic absorption spectrum of ArO• and α-Toc• radicals during the reaction of ArO• with α-TocH in acetonitrile solution at 25.0 °C. Spectra were recorded at 20 ms intervals. The arrow indicates a decrease (ArO•) and an increase (α-Toc•) in absorbance with time. $[\text{ArO}\cdot]_{t=0}$ and $[\alpha\text{-TocH}]_{t=0}$ indicate the concentrations obtained by mixing acetonitrile solutions of α-TocH ($4.80 \times 10^{-3} \text{ M}$) (cell A) and ArO• ($1.25 \times 10^{-4} \text{ M}$) (cell B) (1:1 in volume). (b) Time dependence of the concentration of α-Toc• radical observed, at $\lambda_{\text{max}} = 425.0$ and 430.0 nm, during the reaction of ArO• with α-TocH in acetonitrile solution including 0 M and 0.300 M of LiClO₄, respectively (solid line). The dotted line is a simulation curve.

increasing concentrations of the metal salts (see Figure 3). The values of ϵ were determined, following the method described in section 3.3 and reported in a previous work.¹⁸ As observed for the λ_{max} value of α-Toc•, the ϵ_{max} value also increased, with increasing concentrations of metal salts, and approached different constant values at high concentrations of metal salt, as shown in Figure 5 and as listed in Table 1. However, the reason for the increase of the ϵ value is not clear at present. At the same concentration of metal salts, the ϵ value increased in the following order:

$$\text{without metal salt} < \text{NaClO}_4 < \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2 \quad (7)$$

Measurements of the ϵ values of α-Toc• radical have previously been performed for the absorption spectrum obtained by the reaction of DPPH• with α-TocH in benzene, ethanol, dichloromethane, and *n*-hexane solutions.^{17,29,30}



However, it is not easy to determine the correct ϵ values of α-Toc• because strong absorptions of DPPH• and DPPH₂

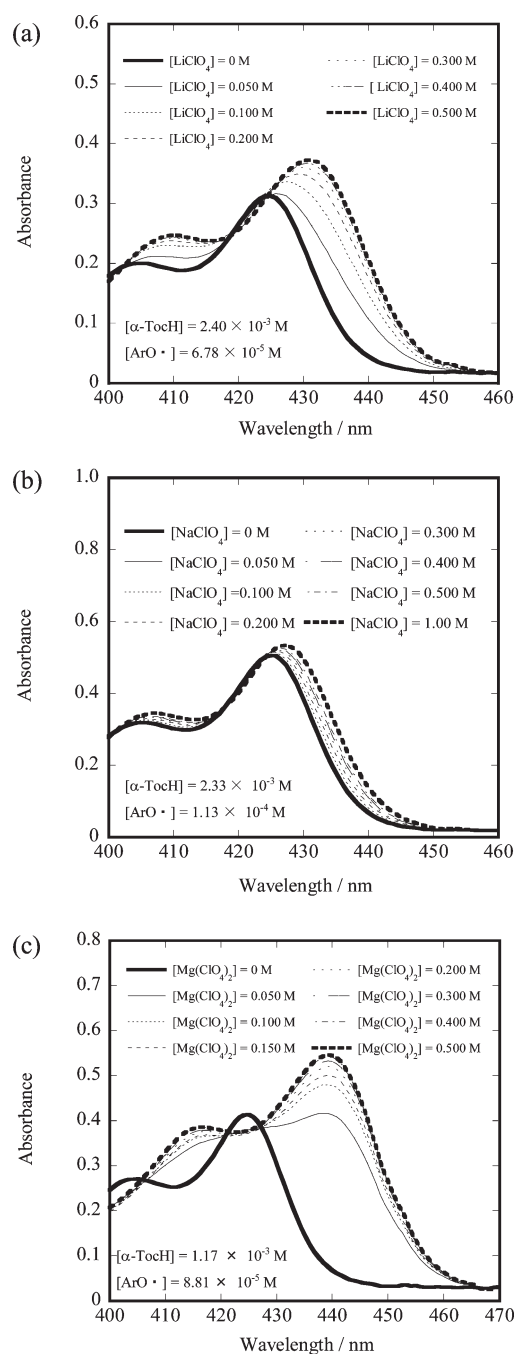


Figure 3. UV–visible absorption spectra at t_{max} for α-Toc• radical at 25.0 °C in acetonitrile solution including various concentrations of metal salts: (a) LiClO₄; (b) NaClO₄; and (c) Mg(ClO₄)₂.

overlap the absorption of α-Toc• in the ~418–428 nm region, and thus, the baseline corrections are necessary. On the other hand, as the absorption of the ArO• and ArOH is negligible in the ~418–428 nm region (see Figure 2a), we can directly determine the ϵ values of α-Toc•, as described above.¹⁸ This is the reason why we chose ArO• radical instead of DPPH• radical to measure the ϵ and $2k_d$ values of α-Toc•.

3.2. Effect of the Metal Salts on the Scavenging Rate (k_s) of Aroxyl Radical by α-Tocopherol in Acetonitrile. Measurements of the rate constant (k_s) for the reaction of ArO• radical with α-TocH were performed in acetonitrile solution at 25.0 °C

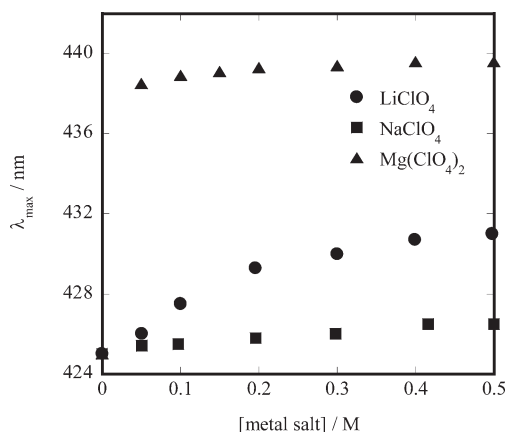


Figure 4. Plots of the peak wavelength (λ_{\max}) of α -Toc $^{\bullet}$ radical vs the concentration of metal salts: (a) LiClO_4 ; (b) NaClO_4 ; and (c) $\text{Mg}(\text{ClO}_4)_2$. Measurements were performed in acetonitrile solution at 25.0 °C.

Table 1. Values of UV–Visible Peak Wavelength (λ_{\max}) and Molar Extinction Coefficients (ϵ_{\max}) of the α -Tocopheroxyl Radical in Acetonitrile Solution Including Different Concentrations of Metal Salts (LiClO_4 , LiI , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$), Stability Constants (K) of the Li^+ , Na^+ , and Mg^{2+} Complexes with α -Tocopheroxyl Radical in Acetonitrile at 25.0 °C, and Ionic Radius (r) of Cations

[salt]/M	$\lambda_{\max}^a/\text{nm}$ ($\epsilon_{\max}^b/\text{M}^{-1}\text{cm}^{-1}$)			
	LiClO_4	LiI	NaClO_4	$\text{Mg}(\text{ClO}_4)_2$
0	425.0 (4320)	425.0	425.0 (4320)	425.0 (4320)
0.050	426.0 (4420)	426.3	425.4 (4350)	438.5 (4360)
0.100	427.5 (4710)	427.4	425.5 (4390)	438.9 (5078)
0.150		428.4		439.1 (5320)
0.200	429.3 (4900)	429.3	425.8 (4440)	439.3 (5540)
0.300	430.0 (5060)	430.5	426.0 (4450)	439.4 (5680)
0.400	430.7 (5170)	430.5	426.5 (4470)	439.6 (5780)
0.500	431.0 (5250)		426.5 (4480)	439.6 (5830)
1.00	insoluble		427.0 (4510)	insoluble
stability constant (K/M^{-1})	9.2		2.8	45
ionic radii of cations $r/\text{\AA}$	0.76	0.76	1.02	0.72

^a Experimental errors are ± 0.5 nm. ^b Experimental errors are $\pm 7\%$. ^c Experimental errors are $\pm 10\%$.

in the presence and absence of three kinds of the metal salts (LiClO_4 , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$). The rate constant (k_s) was determined by analyzing the decay curve of ArO^{\bullet} at 375.0 nm, as reported in previous works.^{18,27} Measurements of the k_s values (7.99×10^3 , 8.00×10^3 , and $8.18 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) without metal salts were repeated three times, and the average value ($\text{av } k_s = 8.06 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) obtained is listed in Table 2. Experimental errors were estimated to be less than 5%.

Similar measurements were performed for the reaction of ArO^{\bullet} with α -TocH in acetonitrile solution, including various concentrations of LiClO_4 , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$ salts, and the k_s values obtained are listed in Table 2. The k_s values

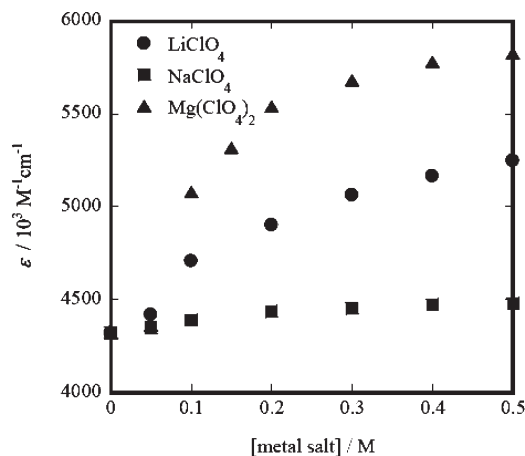


Figure 5. Plots of the molar extinction coefficient (ϵ) of α -Toc $^{\bullet}$ radical vs the concentration of metal salts: (a) LiClO_4 ; (b) NaClO_4 ; and (c) $\text{Mg}(\text{ClO}_4)_2$. Measurements were performed in acetonitrile solution at 25.0 °C.

Table 2. Second-Order Rate Constants (k_s) for the Reaction of α -Tocopherol with ArO^{\bullet} Radical in Acetonitrile Solution Including Different Concentrations of Metal Salts at 25.0 °C and the Ratio ($k_s(\text{with})/k_s(\text{without})$)

[salt]/M	LiClO_4		NaClO_4		$\text{Mg}(\text{ClO}_4)_2$	
	$k_s^a/(\text{M}^{-1} \text{ s}^{-1})$	ratio	$k_s^a/(\text{M}^{-1} \text{ s}^{-1})$	ratio	$k_s^a/(\text{M}^{-1} \text{ s}^{-1})$	ratio
0	8.06×10^3 ^b	1.00	8.06×10^3 ^b	1.00	8.06×10^3 ^b	1.00
0.100					8.55×10^3	1.06
0.140					8.68×10^3	1.08
0.200	8.38×10^3	1.04	8.33×10^3	1.03	9.16×10^3	1.14
0.300	8.53×10^3	1.06				
0.400	8.44×10^3	1.05	8.46×10^3	1.05	9.46×10^3	1.17
0.460					9.98×10^3	1.24
0.600	8.78×10^3	1.09	8.74×10^3	1.08	1.03×10^4	1.28
0.800	insoluble		8.88×10^3	1.10	insoluble	
1.00			9.23×10^3	1.15		

^a Experimental errors are $\pm 5\%$. ^b Average value of k_s obtained without metal salt (see text).

increased with increasing the concentration of metal salts, as shown in Figure 6. Further, the values increased in the following order at the same concentration of metal salts.

$$\text{without metal salt} < \text{NaClO}_4 \sim \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2 \quad (9)$$

The ratios of the k_s values with metal salts to those without the metal salts ($k_s(\text{with})/k_s(\text{without})$) are listed in Table 2.

The rate constants (k_s) obtained in the presence of 0.600 M of NaClO_4 , LiClO_4 , and $\text{Mg}(\text{ClO}_4)_2$ are 1.08, 1.09, and 1.28 times larger, respectively, than those in the absence of metal salts. The reason for this is discussed in the Discussion section. The λ_{\max} value could be obtained for the solution including LiI salt, as described above. However, we were unsuccessful in determining the rate constant (k_s), because ArO^{\bullet} was unstable in acetonitrile solution including LiI salt. The reason is not clear at present.

We tried to measure the rate constants for NaCl , KCl , MgCl_2 , and CaCl_2 , which are included in biological systems.^{19,20} However,

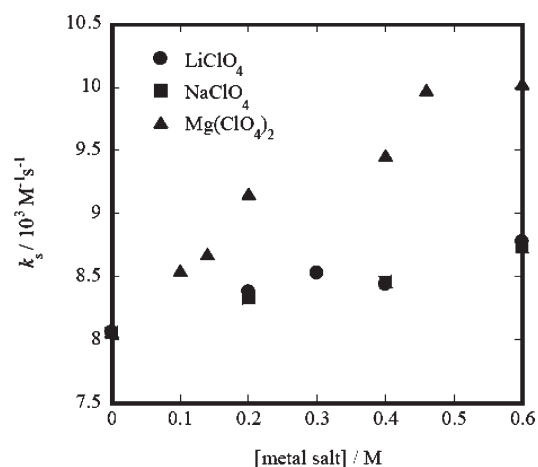


Figure 6. Plots of the second-order rate constant (k_s) for the reaction of α -TocH with the ArO^\bullet radical vs the concentration of metal salts: (a) LiClO_4 ; (b) NaClO_4 ; and (c) $\text{Mg}(\text{ClO}_4)_2$. Measurements were performed in acetonitrile solution at 25.0 °C.

the measurements were unsuccessful because of the low solubility of the salts in acetonitrile.

3.3. Effect of Metal Salts on the Bimolecular Reaction Rate ($2k_d$) of α -Tocopheroxyl Radical in Acetonitrile. Measurement of the formation and decay curves of α -Toc $^\bullet$ radical was performed in acetonitrile solution including three kinds of metal salts (LiClO_4 , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$) by varying the concentrations of the metal salts, to study the effect of metal ions on the bimolecular reaction rates ($2k_d$) and to clarify the mechanism of the bimolecular reaction.

By reacting ArO^\bullet with α -TocH in acetonitrile (reaction 5), α -Toc $^\bullet$ radical is produced rapidly, shows a maximum (at t_{max}), and disappears by the bimolecular self-reaction (reaction 4),^{9,16–18,27} as shown in Figure 2. Independent reaction equations included in reactions 5 and 4 are as follows:

$$-\text{d}[\text{ArO}^\bullet]/\text{d}t = k_s[\alpha\text{-TocH}][\text{ArO}^\bullet] \quad (10)$$

$$\text{d}[\alpha\text{-Toc}^\bullet]/\text{d}t = k_s[\alpha\text{-TocH}][\text{ArO}^\bullet] - 2k_d[\alpha\text{-Toc}^\bullet]^2 \quad (11)$$

In previous works, eqs 9 and 10 were solved numerically using the fourth-order Runge–Kutta method, and the $2k_d$ value was determined for several solvents.^{9,18} Similar analysis was performed in the present work. The simulation of the formation and decay curves of α -Toc $^\bullet$ at $\lambda_{\text{max}} = 425.0$ nm in acetonitrile was performed by varying the rate ($2k_d$) of bimolecular reaction and the molar extinction coefficient (ϵ) at 425.0 nm, where the concentration of α -Toc $^\bullet$ radical at time t , $[\alpha\text{-Toc}^\bullet]_t$, was calculated from the absorbance by using Lambert–Beer's equation (absorbance $A_t = \epsilon[\alpha\text{-Toc}^\bullet]_t$). For comparison, the simulation curve calculated for the case of $2k_d = 0 \text{ M}^{-1} \text{ s}^{-1}$ is also shown in Figure 2b. A good agreement between the observed and simulation curves was obtained for $[\alpha\text{-TocH}] = 2.40 \times 10^{-3} \text{ M}$ at $t = 0$ –10 s, when we used the following values: $k_s = 8.06 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, $2k_d = 1.60 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, and $\epsilon = 4320 \text{ M}^{-1} \text{ cm}^{-1}$ at $\lambda_{\text{max}} = 425.0$ nm (see Figure 2b). The measurements of the $2k_d$ values (1600, 1530, and 1770 $\text{M}^{-1} \text{ s}^{-1}$) without metal salts were repeated three times, and the average value (av $2k_d = 1630 \text{ M}^{-1} \text{ s}^{-1}$) obtained is listed in Table 3. Experimental errors were estimated to be less than 7%.

Table 3. Bimolecular Rate Constants ($2k_d$) in Acetonitrile Solution Including Different Concentrations of Metal Salts at 25.0 °C and the Ratio $2k_d(\text{with})/2k_d(\text{without})$

	LiClO_4		NaClO_4		$\text{Mg}(\text{ClO}_4)_2$	
[salt]/M	$2k_d^a/(\text{M}^{-1} \text{ s}^{-1})$	ratio	$2k_d^a/(\text{M}^{-1} \text{ s}^{-1})$	ratio	$2k_d^a/(\text{M}^{-1} \text{ s}^{-1})$	ratio
0	1630 ^b	1.00	1630 ^b	1.00	1630 ^b	1.00
0.030					650	0.399
0.050	1100	0.674			500	0.307
0.100	750	0.460			350	0.214
0.150					200	0.123
0.200	550	0.337	1280	0.785	200	0.123
0.300	450	0.276			150	0.0920
0.400	400	0.245	1150	0.706	120	0.0736
0.500	400	0.245			120	0.0736
0.600	200	0.123	1030	0.632		
0.800			980	0.601		
1.00			900	0.552		

^a Experimental errors are $\pm 7\%$. ^b Average value of $2k_d$ obtained without metal salt (see text).

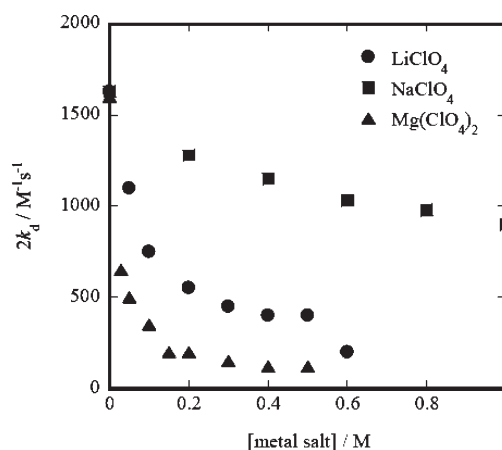


Figure 7. Plots of the second-order rate constant ($2k_d$) for the bimolecular reaction of α -Toc $^\bullet$ radical vs the concentrations of metal salts: (a) LiClO_4 ; (b) NaClO_4 ; and (c) $\text{Mg}(\text{ClO}_4)_2$. Measurements were performed in acetonitrile solution at 25.0 °C.

As shown in Figure 2b, the concentration of α -Toc $^\bullet$ ($[\alpha\text{-Toc}^\bullet]_t = 6.19 \times 10^{-5} \text{ M}$) at $t_{\text{max}} = 0.24 \text{ s}$ is similar to the initial concentration of ArO^\bullet ($[\text{ArO}^\bullet]_{t=0} = 6.25 \times 10^{-5} \text{ M}$), if the concentration of α -TocH is high ($[\alpha\text{-TocH}] = 2.40 \times 10^{-3} \text{ M}$); thus, ArO^\bullet and α -Toc $^\bullet$ rapidly disappear and appear, respectively.¹⁸ Therefore, the value of ϵ is also easily determined from the absorbance (A_{max}) of the α -Toc $^\bullet$ at t_{max} (see Figure 2b and Figure 3). The values of ϵ obtained are listed in Table 1, and the plots of ϵ vs $[\text{MX}]$ are shown in Figure 5.

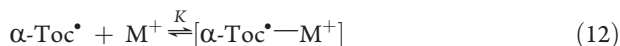
Notable effects of the metal salts were observed for the bimolecular reaction rate of α -Toc $^\bullet$ radical in acetonitrile solvent. As shown in Figure 2b, the $2k_d$ value was $450 \text{ M}^{-1} \text{ s}^{-1}$ when 0.300 M LiClO_4 was included in acetonitrile solution. The $2k_d$ value (av $1630 \text{ M}^{-1} \text{ s}^{-1}$) of α -Toc $^\bullet$ without metal salts decreased, with increasing concentrations of metal salts, as shown in Figure 7.

In the case of NaClO₄ salt, the $2k_d$ value decreased gradually, with increasing concentrations of NaClO₄ salt. On the other hand, in the case of Mg(ClO₄)₂ salt, a rapid decrease of the $2k_d$ value was observed. The decay of the absorption of α -Toc \cdot radical was very slow if high concentrations of Mg(ClO₄)₂ salts (>0.40 M) were included in acetonitrile. The $2k_d$ values of α -Toc \cdot including 0.400 M of metal salts were 1150, 400, and 120 M⁻¹ s⁻¹ for NaClO₄, LiClO₄, and Mg(ClO₄)₂, respectively.

4. DISCUSSION

4.1. Complex Formation between α -Tocopheroxyl Radical and Metal Cations in Acetonitrile Solution. As shown in Figure 4, the λ_{\max} value (425.0 nm) of α -Toc \cdot without metal salts increases, with increasing concentrations of the metal salts in acetonitrile, suggesting the [α -Toc \cdot -M⁺ (or M²⁺)] complex formation between α -Toc \cdot radical and metal cations. The effect of the anion of the metal salts is negligible, because LiClO₄ and LiI show similar shift.

If M⁺ ion forms a (1:1) complex with α -Toc \cdot , the following equilibrium between α -Toc \cdot and M⁺ ion may exist in acetonitrile solution:

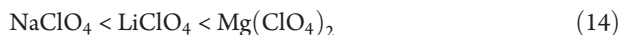


where K is a stability constant for eq 12. With increasing concentrations of the metal salts, the concentrations of the complex ([α -Toc \cdot -M⁺]) will increase gradually; thus, the shifts of the absorption maximum (λ_{\max}) proceed.

The stability constant (K) may be determined using the following equation:³¹

$$(A_0 - A_\infty)/(A_0 - A) = 1 + 1/(K[\text{M}^+]) \quad (13)$$

where A is the absorbance at a fixed wavelength of interest in the 425–440 nm region in the presence of a certain concentration of M⁺ ion. A_0 and A_∞ are the absorbances at the same wavelength in the absence of M⁺ ion and in the presence of a large excess of M⁺ ion, respectively, sufficient to form the complex up to >99%. ($A_0 - A_\infty$)/($A_0 - A$) vs [M^+]⁻¹ plots are shown in Figure 8, suggesting the formation of (1:1) complexes. The stability constants (K) obtained from the slopes in Figure 8 are listed in Table 1. The values increase in the following order:



As reported in a previous work,²⁷ the effect of the metal salts (LiI, LiClO₄, NaI, NaClO₄, KI, and Mg(ClO₄)₂) on the UV–vis absorption spectrum of the α -Toc \cdot radical was negligible in protic methanol solvent, suggesting that the complex formations between α -Toc \cdot radical and metal cations are hindered by the hydrogen bond between α -Toc \cdot radical and methanol molecules. On the other hand, in the present study, a notable effect of the metal salts (LiClO₄, LiI, NaClO₄, and Mg(ClO₄)₂) on the UV–vis absorption spectrum was observed in aprotic acetonitrile solvent.

The formation of a [α -Toc \cdot -M⁺ (or M²⁺)] complex will be due to the Coulomb interaction between the metal cation (M⁺ or M²⁺) and O₆ (and/or O₁) atom (having negative charge density) in an α -Toc \cdot radical molecule. The ionic radii (r) of Li⁺, Na⁺, and Mg²⁺ (the values for six coordinations) are 0.76, 1.02, and 0.72 Å, respectively. By comparing the λ_{\max} values of LiClO₄ and NaClO₄, the former, having a smaller ionic radius, shows larger

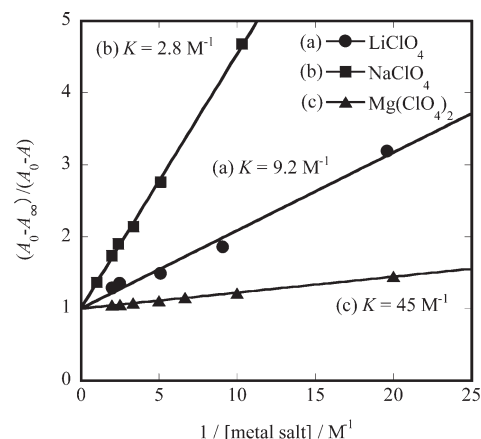
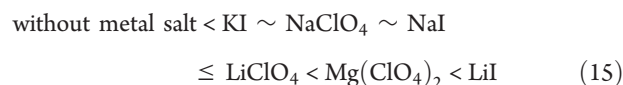


Figure 8. Plots of $(A_0 - A_\infty)/(A_0 - A)$ vs the inverse concentration of metal salts ($[\text{metal salt}]^{-1}$): (a) LiClO₄; (b) NaClO₄; and (c) Mg(ClO₄)₂. Measurements were performed in acetonitrile at 25.0 °C.

shift than the latter. The ionic radius of Mg²⁺ (0.72 Å) is similar to that of Li⁺ (0.76 Å). However, the shift of λ_{\max} value for Mg(ClO₄)₂ is larger than that for LiClO₄. The result indicates that the Mg²⁺ ion having smaller ionic radius and larger positive charge of +2e gives larger shift in λ_{\max} value and larger K value.

4.2. Mechanism of the Aroxyl Radical-Scavenging Reaction of α -Tocopherol in Acetonitrile: Effect of the Metal Salts on the Reaction Rates (k_s). As described in the Introduction, Nakanishi et al.^{23,24} measured the reaction rates of an α -tocopherol model with galvinoxyl \cdot (and DPPH \cdot) radicals in methanol including Mg(ClO₄)₂ salt and found that the rate constants (k_s) increase, with increasing concentrations of Mg(ClO₄)₂. On the other hand, no effect of Mg(ClO₄)₂ on the rate constants (k_s) was observed in acetonitrile. In our previous work,²⁷ measurement of the rate constant (k_s) for the reaction of ArO \cdot with α -TocH was performed in protic methanol solution including six kinds of metal salts. The k_s values increased in the following order:



The result suggests that reaction 5 proceeds via an electron-transfer intermediate [$\text{ArO}\cdot^-$ - α -TocH^{•+}] in methanol (see Figure 9a).

In the present work, the measurement of the rate constant (k_s) was performed in aprotic acetonitrile solution including LiClO₄, NaClO₄, and Mg(ClO₄)₂ salts. As shown in Figure 6, the k_s values increased in the order of eq 9. The order of eq 9 is the same as that of eq 15, suggesting that reaction 5 in acetonitrile also proceeds via an electron-transfer intermediate. As shown in Figure 9a, if the transition state of the above ArO \cdot -scavenging reaction by α -TocH has the property of an electron-transfer intermediate [$\text{ArO}\cdot^-$ - α -TocH^{•+}], the metal cations (Li⁺, Na⁺, and Mg²⁺) will interact with the ArO \cdot^- anion by Coulomb interaction and form a complex with the ArO \cdot^- anion [$\text{ArO}\cdot^-$ -M⁺ (or M²⁺)].^{24,27} Similarly, the ClO₄⁻ anion of the salts will also interact with α -TocH^{•+} cation and form a complex with the α -TocH^{•+} cation [α -TocH^{•+}-ClO₄⁻]. As a result, the energy level of an electron-transfer intermediate [$\text{ArO}\cdot^-$ - α -TocH^{•+}] will be stabilized (that is, the activation energy (E_{act}) will decrease), and thus, the k_s values increase (see Figure 9a). The Coulomb interaction energy will increase, with decreasing ionic radii of

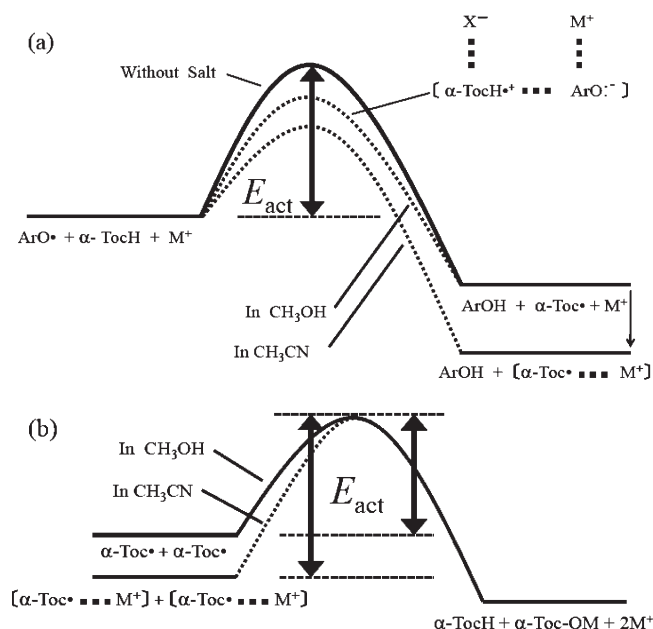


Figure 9. (a) Mechanism of the ArO^\bullet radical-scavenging reaction of $\alpha\text{-TocH}$. The transition state for the reaction of ArO^\bullet with $\alpha\text{-TocH}$ has the property of an electron-transfer intermediate $[\text{ArO}^\bullet - \alpha\text{-TocH}^\bullet]$, and its energy level is lowered by the complex formations between (i) ArO^\bullet anion and M^+ cation and (ii) $\alpha\text{-TocH}^\bullet$ cation and X^- anion. The energy level of the reaction products (ArOH and $\alpha\text{-Toc}^\bullet$) is lowered by the complex formation between $\alpha\text{-Toc}^\bullet$ and metal cations (M^+ (or M^{2+})) (see text). (b) Mechanism of the bimolecular self-reaction of $\alpha\text{-Toc}^\bullet$ radical. The energy level of the reactants ($\alpha\text{-Toc}^\bullet + \alpha\text{-Toc}^\bullet$) is lowered by the complex formation between $\alpha\text{-Toc}^\bullet$ and metal cations (M^+ (or M^{2+})) (see text).

cations (Na^+ , Li^+ , and Mg^{2+}) and with increasing charges of cations from +1e to +2e. In fact, the rate constants (k_s) increase in the order of eq 8.

As described in the Results section, the changes of the UV–vis absorption spectra of ArO^\bullet , ArOH , and $\alpha\text{-TocH}$ were negligible in acetonitrile in the presence of three metal salts (0.400 M), showing that the interactions between these neutral molecules and the cations (and anions) of metal salts are negligibly weak. On the other hand, a notable red shift of the λ_{max} value of the absorption spectrum of the $\alpha\text{-Toc}^\bullet$ was observed, indicating the complex formation between $\alpha\text{-Toc}^\bullet$ and metal cations. The results suggest that the total energy level of the reaction products ($\text{ArOH} + \alpha\text{-Toc}^\bullet$) in reaction 5 may be stabilized by the complex formation $[\alpha\text{-Toc}^\bullet - \text{M}^+ \text{ (or } \text{M}^{2+})]$ (see Figure 9a). On the other hand, the total energy level of the reactants ($\text{ArO}^\bullet + \alpha\text{-TocH}$) does not vary in the presence of metal salts. In such a case, the increase of the rate constants (k_s) will be observed in the presence of metal salts, as expected from the Evans–Polanyi theory.^{32,33} In fact, the reaction rate constants (k_s) showed considerable increase in the presence of three metal salts (see Figure 6).

The results indicate that not only (i) the stabilization of the energy level at the transition intermediate but also (ii) the stabilization of the energy level of the reaction products ($\text{ArOH} + \alpha\text{-Toc}^\bullet$) may contribute to the increase of the rate constant (k_s) in acetonitrile. However, it is not clear at the present which stabilization effects contribute more effectively to the increase of the rate constant (k_s) in acetonitrile.

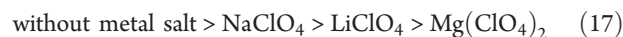
4.3. Mechanism of the Bimolecular Self-Reaction of $\alpha\text{-Toc}^\bullet$ Radical: Effect of Metal Salts on the Reaction Rates ($2k_d$). As described in the Results section, the decay of the $\alpha\text{-Toc}^\bullet$ radicals in acetonitrile follows the bimolecular self-reaction of $\alpha\text{-Toc}^\bullet$ radicals, suggesting the production of $\alpha\text{-TocH}$ and $\alpha\text{-tocopherol-}o\text{-quinonemethide}$ ($\alpha\text{-Toc-QM}$) (see Figure 1) by disproportionation reaction.^{16–18}



Nakanishi et al.^{23,24} studied the effect of $\text{Mg}(\text{ClO}_4)_2$ salt on the decay rate ($2k_d$) of the $\alpha\text{-Toc}^\bullet$ model radical in acetonitrile and found that the rate constant ($2k_d = 2.7 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) in the absence of metal salt decreases remarkably in the presence of 0.1 M $\text{Mg}(\text{ClO}_4)_2$. Further, they reported that the maximum wavelength ($\lambda_{\text{max}} = 423 \text{ nm}$) of the UV–vis absorption spectrum of the $\alpha\text{-Toc}^\bullet$ model shifts to $\lambda_{\text{max}} = 437 \text{ nm}$ in the presence of 0.1 M $\text{Mg}(\text{ClO}_4)_2$, suggesting $[\alpha\text{-Toc}^\bullet - \text{Mg}^{2+}]$ complex formation. It has been reported that such a complex formation may stabilize the energy level of the reactant in reaction 16, resulting in the increase of the activation energy (E_{act}).

In a previous work,²⁷ we studied the effect of six metal salts (LiI , LiClO_4 , NaI , NaClO_4 , KI , and $\text{Mg}(\text{ClO}_4)_2$) on the UV–vis absorption spectrum of $\alpha\text{-Toc}^\bullet$ radical in methanol solution. However, the shift of the absorption peak of $\alpha\text{-Toc}^\bullet$ at $\lambda_{\text{max}} = 429 \text{ nm}$ was not observed in methanol in the presence of all the metal salts used. Further, the effect of metal salts on the rate constants ($2k_d$) of the bimolecular reaction of $\alpha\text{-Toc}^\bullet$ radical was not observed in protic methanol solution.²⁷ If the electron transfer intermediate having a structure of $[\alpha\text{-Toc}^\bullet - \alpha\text{-Toc}^\bullet]$ contributed to the reaction, the rate constant ($2k_d$) should increase in the presence of metal salts, as observed for the reaction between ArO^\bullet and $\alpha\text{-TocH}$. The result indicates that the hydrogen transfer between two $\alpha\text{-Toc}^\bullet$ radical molecules proceeds via a direct one-step hydrogen atom transfer rather than via electron transfer. That is to say, the electron transfer between two $\alpha\text{-Toc}^\bullet$ radical molecules having the same electronic structure seems to be a difficult occurrence.

On the other hand, in the present work, a notable effect of metal salts (LiClO_4 , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$) on the rate constants ($2k_d$) was observed in aprotic acetonitrile solvent. The rate constants ($2k_d$) of the bimolecular reaction of $\alpha\text{-Toc}^\bullet$ radical decreased notably, with increasing concentrations of metal salts, as shown in Figure 7 and listed in Table 3. Further, the $2k_d$ value decreased at the same concentration of metal salts in the following order:



The decrease of the $2k_d$ value will be due to the complex formation between $\alpha\text{-Toc}^\bullet$ radical and metal cations.

As described in the Results section, a notable red shift of the λ_{max} value of the absorption spectrum of the $\alpha\text{-Toc}^\bullet$ was observed in the presence of the metal salts in acetonitrile, indicating the complex formation between $\alpha\text{-Toc}^\bullet$ and metal cations (Li^+ , Na^+ , and Mg^{2+}). On the other hand, the shift of the λ_{max} value of $\alpha\text{-TocH}$ was not observed in acetonitrile in the presence of the metal salts, showing that the interactions between $\alpha\text{-TocH}$ and the cations and anions of metal salts are negligibly weak. Similarly, the interactions between metal salts and $\alpha\text{-Toc-QM}$ will be small and negligible, although we could not ascertain it, because $\alpha\text{-Toc-QM}$ is unstable and not isolable. The results suggest that the total energy level of the reactants ($\alpha\text{-Toc}^\bullet + \alpha\text{-Toc}^\bullet$) in reaction 16

will be lowered by the complex formation (see Figure 9b). On the other hand, the total energy level of the products (α -TocH + α -Toc-QM) does not vary in the presence of metal salts. In such a case, the decrease of the reaction rate constant ($2k_d$) will be observed in the presence of metal salts, as expected from the Evans–Polanyi theory.^{32,33}

The difference in the rate constants for NaClO_4 , LiClO_4 , and $\text{Mg}(\text{ClO}_4)_2$ will be explained as follows: as the stability constant (K) increases in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2$, the stabilization energy of the reactants (α -Toc $^\bullet$ + α -Toc $^\bullet$) will increase in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2$. Consequently, the activation energy for the bimolecular reaction 16 increases in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2$, resulting in the decrease in the $2k_d$ value in the order $\text{NaClO}_4 > \text{LiClO}_4 > \text{Mg}(\text{ClO}_4)_2$ (see Figure 9b).

4.4. Alkali and Alkaline Earth Metal Salts May Affect Free Radical-Scavenging and Prooxidant Reactions of α -Tocopherol in Food and Biological Systems. As described in the Introduction, α -TocH and alkali and alkaline earth metal ions (Na^+ , K^+ , Mg^{2+} , and Ca^{2+}) coexist in blood, plasma, plasma lipoproteins, and cellular membranes.^{19–22} For instance, it was reported that high concentrations of Na^+ and K^+ ions are included in human blood ($[\text{Na}^+] = 81.7 \text{ mM}$ and $[\text{K}^+] = 44.6 \text{ mM}$) and plasma ($[\text{Na}^+] = 136 \text{ mM}$ and $[\text{K}^+] = 3.6 \text{ mM}$).¹⁹ The concentrations of metal cations found in mammals are $[\text{Na}^+] = 140 \text{ mM}$, $[\text{K}^+] = 4 \text{ mM}$, and $[\text{Mg}^{2+}] = 1.5 \text{ mM}$ in extracellular fluid and $[\text{Na}^+] = 10 \text{ mM}$, $[\text{K}^+] = 140 \text{ mM}$, and $[\text{Mg}^{2+}] = 30 \text{ mM}$ in intracellular fluids.³⁴ The concentration of α -TocH in human plasma was reported to be on average 22.0 (12.0 – 36.0) μM .²¹ Therefore, the concentrations of Na^+ , K^+ , and Mg^{2+} ions are much higher than that of α -TocH in human plasma.

Na^+ ion has the highest concentration ($[\text{Na}^+] = 136 \text{ mM}$) among alkali and alkaline earth metal ions in plasma. As shown in Figures 6 and 7 and listed in Tables 2 and 3, the k_s and $2k_d$ values in acetonitrile with 136 mM of Na^+ ion are $\sim 2\%$ larger and $\sim 15\%$ smaller than those without Na^+ ion, respectively. Similarly, the k_s and $2k_d$ values in acetonitrile with 30 mM Mg^{2+} ion are $\sim 2\%$ larger and $\sim 60\%$ smaller than those without Mg^{2+} ion, respectively. The effects of Na^+ and Mg^{2+} ions on the free radical-scavenging reaction rate constants (k_s) are small and negligible. On the other hand, the effects of Na^+ and Mg^{2+} ions on the bimolecular self-reaction rate ($2k_d$) are remarkable.

As described in the Introduction, α -Toc $^\bullet$ radicals are important key radicals, which appear in the process of the antioxidant and prooxidant actions of α -TocH (see reactions 1–5). In the present work, detailed kinetic studies have been performed for the bimolecular reaction 4 and for the free radical-scavenging reaction 5, and the mechanisms involved and the effect of metal salts on the reaction rates ($2k_d$ and k_s) have been studied.

As described in section 4.1, α -Toc $^\bullet$ radical molecule forms a (1:1) complex with metal cations in acetonitrile. When α -Toc $^\bullet$ form complexes with metal cations (such as Na^+ , K^+ , Mg^{2+} , and Ca^{2+} ions), the total energy levels of the reactants (α -Toc $^\bullet$ + LH in reaction 2^{5–9} and α -Toc $^\bullet$ + AsH^- (or UQ_{10}H_2) in reaction 3^{10–15}) will decrease, and thus, the k_p and k_t values will decrease.^{32,33} That is, the prooxidant effect of α -TocH, which is observed in edible oils and foods that include a high concentration of α -TocH, may be suppressed by the addition of the metal salts (NaCl , KCl , MgCl_2 , and CaCl_2). On the other hand, the rate constants for the regeneration reaction of α -Toc $^\bullet$ by vitamin C and ubiquinol-10 in biomembranes will also decrease under the existence of the metal salts. We can expect notable effects of

metal salts on the biologically important reactions 2 and 3. It will be interesting to investigate the effects of metal salts on the reaction rates (k_p and k_t) in solution.

Hydrophilic alkali and alkaline earth metal salts are soluble in methanol and acetonitrile, and they may coexist with the polar OH group of α -TocH at the polar region of the membrane having a polarity similar to that of ethanol.^{27,35–37} The result of the present work suggests that the alkali and alkaline earth metal salts may contribute to reactions 2–4 in biological systems.

5. SUMMARY

In the present work, we studied the effect of four metal salts (LiClO_4 , LiI , NaClO_4 , and $\text{Mg}(\text{ClO}_4)_2$) on (i) the UV–vis absorption spectra of α -Toc $^\bullet$ radical, (ii) bimolecular self-reaction of α -Toc $^\bullet$ radical, and (iii) ArO^\bullet radical-scavenging reaction by α -TocH in acetonitrile solution, using stopped-flow spectrophotometry. Measurements were performed by varying the concentration of metal salts. The maximum wavelength (λ_{max}) of the absorption spectrum of the α -Toc $^\bullet$ at 425.0 nm increased, with increasing concentrations of metal salts (0 – 0.500 M), and approached the constant values, suggesting the complex formation between α -Toc $^\bullet$ radical and metal cations. The stability constants (K) for the complex formation increased in the order $\text{NaClO}_4 < \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2$. The rate constants ($2k_d$) of a bimolecular self-reaction between two α -Toc $^\bullet$ radical molecules (that is, the decay reaction of α -Toc $^\bullet$ radical) decreased notably, with increasing concentrations of metal salts, and they decreased in the following order at the same concentration of metal salts: no metal salt $> \text{NaClO}_4 > \text{LiClO}_4 > \text{Mg}(\text{ClO}_4)_2$. The decrease of the $2k_d$ value will be due to the complex formation between α -Toc $^\bullet$ and metal cations. On the other hand, the second-order rate constants (k_s) for the reaction of ArO^\bullet radical with α -TocH increased, with increasing concentrations of metal salts, and they increased in the following order at the same concentration of metal salts: no metal salt $< \text{NaClO}_4 \sim \text{LiClO}_4 < \text{Mg}(\text{ClO}_4)_2$. The alkali and alkaline earth metal salts having smaller ionic radii of cations and larger cation charges gave larger K and k_s values and a smaller $2k_d$ value in acetonitrile solution. From the results, the mechanisms for the above reactions were discussed.

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