THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 266, No. 1, Issue of January 5, pp. 274-278, 1991
Printed in U.S.A.

Stopped-flow Investigation of the Reaction of Vitamin C with Tocopheroxyl Radical in Aqueous Triton X-100 Micellar Solutions

THE STRUCTURE-ACTIVITY RELATIONSHIP OF THE REGENERATION REACTION OF TOCOPHEROL BY VITAMIN C*

(Received for publication, May 8, 1990)

Kazuo Mukai‡, Motoi Nishimura, and Seiji Kikuchi

From the Department of Chemistry, Faculty of Science, Ehime University, Matsuyama 790, Japan

A kinetic study of the reaction between vitamin C (L-ascorbic acid, AsH_2) and a tocopheroxyl radical (7-*tert*-butyl-5-isopropyltocopheroxyl) in Triton X-100 micellar solution has been performed using stopped-flow spectrophotometry. The second-order rate constants (k_2) obtained showed notable pH dependence with a broad maximum around pH 8. For instance, the k_2 values obtained were 26 M^{-1} s^{-1} at pH 3, 322 M^{-1} s^{-1} at pH 7, and 273 M^{-1} s^{-1} at pH 10. A good correlation between the rate constants and the mole fraction of ascorbate monoanion (AsH^-) was observed, showing that ascorbate (AsH^-) can regenerate the tocopherol from tocopheroxyl in biological systems. Furthermore, the results indicate that reduced ascorbic acid (AsH_2) does not have the ability to regenerate the tocopherol in aqueous solution. On the other hand, it was found that AsH_2 can reduce the tocopheroxyl to tocopherol in benzene/ethanol (2:1) mixtures, although the rate of reaction is only ~15% of that observed in micellar solution at pH 7.

It is well known that tocopherols (vitamin E) are localized in biomembranes and function as efficient inhibitors of lipid peroxidation. The antioxidant action of tocopherols (TocH) has been ascribed to the oxidation of the phenolic hydroxyl group to produce the corresponding tocopheroxyl radicals (Toc·) (Reaction I) (1, 2).

$$LOO^{\cdot}{}^{1} + TocH \xrightarrow{k_1} LOOH + Toc^{\cdot} \quad (I)$$

Tappel (3) made the important suggestion that vitamin C (Fig. 1, ascorbic acid 1) may reduce the tocopheroxyl radical back to the starting tocopherol. Subsequent *in vitro* experiments (4, 5) showed that vitamin C can indeed reduce the α-tocopheroxyl radical (Reaction II).

$$Toc^{\cdot} + Vit\ C \xrightarrow{k_2} TocH + Vit\ C^{\cdot} \quad (II)$$

Packer *et al.* (4) have reported the absolute second-order rate constants (k_2) for the reaction of vitamin C (sodium ascorbate) with α-tocopheroxyl radicals using pulse radiolysis methodology. They found a k_2 value of 1.55×10^6 M^{-1} s^{-1} in a system composed of an aerated, aqueous solution containing isopropyl alcohol (50%) and acetone (10%). Furthermore, Scarpa *et al.* (6) measured the second-order rate constants for the reaction between the α-tocopheroxyl radical in dimyristoylphosphatidylcholine liposomes and vitamin C in the surrounding aqueous phase (pH 7) using an EPR technique. The kinetic rate constant obtained was $\sim 2 \times 10^5$ M^{-1} s^{-1}. This value is only an order of magnitude lower than that reported by Packer *et al.* (4) for the same reaction in homogeneous solution. However, detailed kinetic studies of the interaction between vitamin C derivatives and tocopheroxyl radicals (that is, the regeneration reaction of tocopherol by vitamin C) have not been performed.

Recently, we measured the second-order rate constants for the reaction of 10 kinds of vitamin C derivatives with a vitamin E radical (5,7-diisopropyltocopheroxyl) in organic solvent (benzene/ethanol (2:1, v/v)) using a stopped-flow technique (7, 8). Whereas fatty acid esters of ascorbic acid 1 at the 6-position reacted at rates ~20% faster than ascorbic acid 1 itself, fatty acid esters at both the 5- and 6-positions reacted at rates that were only ~60–80% of that observed for ascorbic acid 1, a finding that may arise from steric hindrance. The octadecyl ether of ascorbic acid 1 at the 2-position (CV-3611) is 0.40 times as reactive as ascorbic acid 1, whereas the ascorbic acid ester substituted at both the 2- and 6-positions is ~0.01 times as reactive as ascorbic acid 1. From these results, the structure-activity relationship for the regeneration of tocopherol by vitamin C in organic solvent systems was discussed. In organic solvents, ascorbic acid 1 is thought to exist in the reduced form (AsH_2), as shown in Fig. 1. On the other hand, ascorbic acid 1 is a dibasic acid and thus, in an aqueous solution system, can exist in three different molecular forms (AsH_2, AsH^-, or As^{2-}) depending on the pH value (see Fig. 1). However, it is not clear at present which form of ascorbic acid 1 is most effective for the regeneration reaction of vitamin E.

Therefore, to clarify the mechanism of the reaction of vitamin C with tocopheroxyl radicals, we have measured the second-order rate constants (k_2) for the reaction of vitamin C with the tocopheroxyl radical (Fig. 1, 7-*tert*-butyl-5-isopropyltocopheroxyl 2) in Triton X-100 micellar solution using a stopped-flow technique and studied the effect of pH on the reaction rate. Furthermore, similar measurements have been made for the reaction of tocopheroxyl with sodium ascorbate (Na^+AsH^-) in Triton X-100 micellar solution. The reaction rate of tocopheroxyl 2 with ascorbic acid 1 has also been measured in benzene/ethanol (2:1, v/v) solution. Based on the results, the structure-activity relationship for the regeneration reaction of tocopherol by vitamin C is discussed.

EXPERIMENTAL PROCEDURES

Commerical L-ascorbic acid 1 and sodium L-ascorbate were used as received. 7-*tert*-Butyl-5-isopropyltocopherol was prepared accord-

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed.

[1] The abbreviations used are: LOO·, lipid peroxyl radical; LOOH, lipid hydroperoxide.

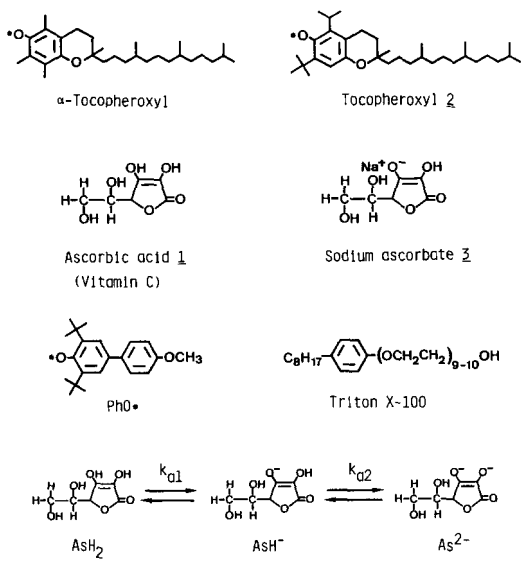

FIG. 1. **Molecular structures of ascorbic acid 1 (AsH_2, AsH^-, and As^{2-}), sodium ascorbate 3 (Na^+AsH^-), α-tocopheroxyl, tocopheroxyl 2, and substituted phenoxyl radical (PhO•).**

ing to the method reported in a previous paper (9). The 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl ("substituted phenoxyl" (PhO•)) was prepared according to the method of Müller *et al.* (10). Triton X-100 was purchased from Nacalai Tesque Inc. (Kyoto, Japan) and used as received. All buffer solutions were prepared using distilled water treated with a Millipore Q system. The pH of the solutions was adjusted using an appropriate buffer (0.1 M): pH 3.0–5.5, CH_3COOH–CH_3COONa; pH 6.0–9.0, KH_2PO_4–$NaHPO_4$; and pH 10.0, $NaHCO_3$–Na_2CO_3.

Tocopheroxyl 2-containing micellar solutions of Triton X-100 (10 weight %) were prepared as follows. 7-*tert*-Butyl-5-isopropyltocopherol (15–20 mg, 31–41 μmol) was dissolved in 5 ml of diethyl ether, and the solution was poured into a small flask. The diethyl ether was removed by evacuation in a water aspirator using a rotary vacuum evaporator to obtain a thin film on the flask wall. Twenty ml of aqueous Triton X-100 micellar solution (10.0 weight %) (0.1 M phosphate buffer (pH 7)) was added, and the flask was shaken vigorously in a Vortex mixer for 30 s. PhO•-containing solutions of Trixon X-100 (10.0 weight %) were prepared similarly and reacted with the above tocopherol-containing micellar solution.

The PhO• radical is very stable in the absence of 7-*tert*-butyl-5-isopropyltocopherol and shows absorption peaks with λ_{max} = 377 and 577 nm in aqueous Triton X-100 micellar solutions (10.0 weight %). Upon mixing the micellar solution of tocopherol (0.28 mM) with the micellar solution of the PhO• radical (0.24 mM) (1:1, v/v) at 25.0 °C, the absorption spectrum of the PhO• radical immediately changes to that of tocopheroxyl 2 (see Fig. 2). The new absorption maxima in the visible region (λ_{max} = 396 and 416 nm) are due to tocopheroxyl 2 (11). Since tocopheroxyl 2 is quite stable at 25.0 °C, the absorption intensity decreases only gradually with time.

Stopped-flow data were obtained on a UNISOKU stopped-flow Model RS-450 spectrophotometer by mixing equal volumes of aqueous Triton X-100 micellar solutions of tocopheroxyl and aqueous buffered solutions of vitamin C (ascorbic acid 1). The oxidation reactions were studied under pseudo first-order conditions, and the observed rate constants (k_{obs}) were calculated in the usual way using standard least-squares analysis. All measurements were performed at 25.0 ± 0.5 °C. As reported in a previous paper (12), α-tocopheroxyl is not stable; thus, the stable 7-*tert*-butyl-5-isopropyltocopheroxyl radical 2 was used for this work.

RESULTS

7-*tert*-Butyl-5-isopropyltocopheroxyl 2 is comparatively stable in the absence of ascorbic acid 1 and shows absorption peaks with λ_{max} = 416 and 396 nm in aqueous Triton X-100 micellar solution (10.0 weight %; 0.1 M phosphate buffer (pH 7.0)) (see Fig. 2). Upon addition of excess ascorbic acid 1 in 0.1 M phosphate buffer to Triton X-100 micellar solution

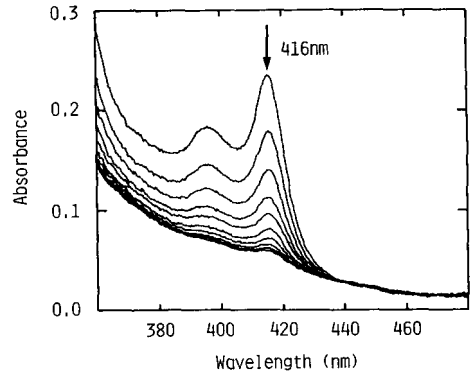

FIG. 2. **Change in electronic absorption spectrum of 7-*tert*-butyl-5-isopropyltocopheroxyl radical 2 during reaction of tocopheroxyl 2 with vitamin C in Triton X-100 micellar solution (0.1 M phosphate buffer (pH 7.0)) at 25.0 °C.** $[Toc^\bullet]_{t=0}$ ~ 0.06 mM, and $[Vitamin\ C]_{t=0}$ = 1.15 mM. The spectra were recorded at 1000-ms intervals. The *arrow* indicates the decrease in absorbance maximum with time.

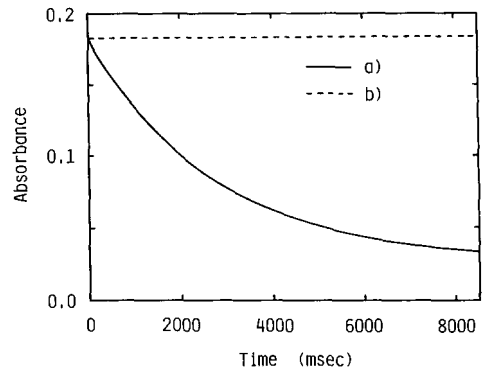

FIG. 3. *Trace a,* the decay of tocopheroxyl radical 2 during the reaction of tocopheroxyl 2 with vitamin C in Triton X-100 micellar solution at 25.0 °C. $[Toc^\bullet]_{t=0}$ ~ 0.09 mM, and $[Vitamin\ C]_{t=0}$ 1.15 mM. The decrease in the absorbance at 416 nm is shown. *Trace b,* the natural decay of tocopheroxyl 2 in the absence of vitamin C in Triton X-100 micellar solution.

containing tocopheroxyl 2 (1:1, v/v), the absorption spectrum of tocopheroxyl 2 disappears immediately. Fig. 2 shows an example of the interaction between tocopheroxyl 2 (~0.06 mM) and ascorbic acid 1 (1.15 mM) in phosphate buffer (pH 7.0). The time course of the decrease in absorbance at 416 nm observed when Triton X-100 micellar solution (phosphate buffer (pH 7.0)) containing tocopheroxyl 2 (~0.09 mM) is mixed with an aqueous solution (phosphate buffer (pH 7.0)) of ascorbic acid 1 (2.30 mM) (1:1, v/v; final concentration of ascorbic acid 1 of 1.15 mM) is shown in Fig. 3. The pseudo first-order rate constants (k_{obs}) obtained by varying the concentration of ascorbic acid 1 are presented in Table I. As shown in Fig. 3, 7-*tert*-butyl-5-isopropyltocopheroxyl 2 shows a very slow natural decay in Triton X-100 micellar solution. Therefore, the pseudo first-order rate constant for tocopheroxyl bleaching is given in Equation 1:

$$k_{obs} = k_0 + k_2\,[Vit\ C] \qquad (1)$$

where k_0 is the rate constant for the natural decay of tocopheroxyl 2 in the medium, and k_2 is the second-order rate constant for the reaction of tocopheroxyl 2 with added ascorbic acid 1. These rate parameters are obtained by plotting k_{obs} against [vitamin C], as shown in Fig. 4. The second-order rate constant obtained for ascorbic acid 1 at pH 7.0 is 322 $M^{-1}\ s^{-1}$ and k_0 = 0.007 s^{-1}.

TABLE I

Pseudo first-order (k_{obs}) and second-order (k_2) rate constants for the reaction of vitamin C (ascorbic acid 1) with tocopheroxyl radical 2 in Triton X-100 micellar solution and the mole fraction (f) of ascorbate monoanion (AsH^-)

pH	[Vitamin C]	k_{obs}	k_2	f ($[AsH^-]/C_a$)
	mM	s^{-1}	$M^{-1}\,s^{-1}$	
3.0	1.02	0.028		
	1.97	0.057		
	2.93	0.083	25.7	0.063
	3.92	0.107		
	5.02	0.131		
3.5	1.08	0.069		
	2.02	0.124		
	3.11	0.185	56.4	0.176
	4.05	0.237		
	4.97	0.289		
4.0	1.04	0.161		
	2.03	0.288		
	3.13	0.428	123	0.403
	4.02	0.533		
	5.06	0.657		
4.5	1.00	0.216		
	2.09	0.434		
	3.06	0.592	188	0.681
	4.11	0.808		
	4.95	0.962		
5.0	1.01	0.260		
	2.14	0.533		
	3.03	0.760	248	0.871
	3.94	0.982		
	4.97	1.24		
5.5	1.16	0.324		
	2.21	0.594		
	3.09	0.834	268	0.955
	4.04	1.10		
	4.95	1.33		
6.0	1.22	0.387		
	2.28	0.726		
	2.97	0.931	311	0.985
	3.94	1.24		
	4.76	1.49		
7.0	1.15	0.374		
	2.25	0.740		
	2.89	0.941	322	0.999
	4.00	1.28		
	4.70	1.53		
8.0	1.02	0.333		
	1.98	0.641		
	3.01	0.986	319	1.000
	3.85	1.25		
	4.70	1.50		
9.0	1.08	0.364		
	2.08	0.692		
	2.98	0.979	314	0.997
	3.94	1.29		
	4.96	1.58		
10.0	1.03	0.312		
	2.09	0.584		
	3.13	0.891	273	0.974
	4.02	1.13		
	5.09	1.41		
Sodium ascorbate	1.06	0.316		
	2.05	0.596		
	2.85	0.827	300[a]	
	3.98	1.19		
	5.21	1.55		
Benzene/ethanol	1.28	0.201		
(2:1, v/v)	1.92	0.232	49[b]	
	2.31	0.251		

[a] The k_2 value obtained for sodium ascorbate (Na^+AsH^-).
[b] The k_2 value obtained in benzene/ethanol (2:1, v/v) solution.

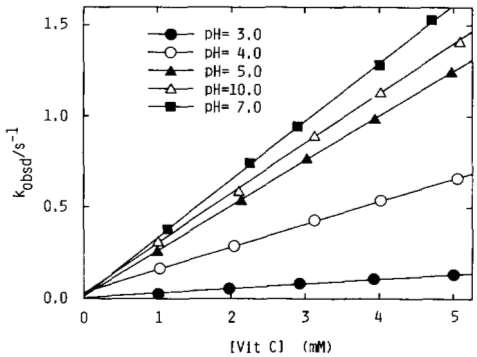

FIG. 4. **Dependence of pseudo first-order rate constants (k_{obs}) on concentration of vitamin C at several pH values in Triton X-100 micellar solution.**

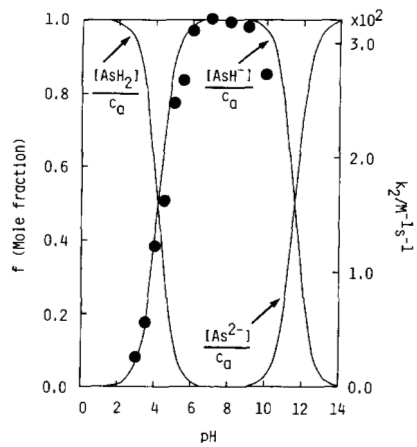

FIG. 5. **Plots of second-order rate constant (k^2) *versus* pH and of mole fraction (f) of three vitamin C species (AsH_2, AsH^-, and As^{2-}) *versus* pH.**

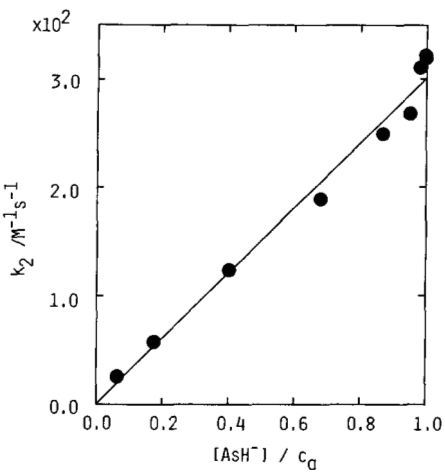

FIG. 6. **Plot of second-order rate constant (k_2) *versus* mole fraction of ascorbate monoanion ($f = [AsH^-]/C_a$).**

Similar measurements were performed for the reaction of tocopheroxyl 2 with ascorbic acid 1 at various pH values. The reaction rate for sodium ascorbate 3 (Na^+AsH^-) with tocopheroxyl 2 in Triton X-100 micellar solution was also measured. The k_2 values obtained are summarized in Table I. Where pH is <3 or >11, tocopheroxyl radical 2 is unstable, and the measurement of the k_2 values was unsuccessful. The pH dependence of the second-order rate constants is shown in Fig. 5. When the pH values are >3, the k_2 increases rapidly from 26 $M^{-1}\,s^{-1}$ at pH 3.0 to 311 $M^{-1}\,s^{-1}$ at pH 6.0, remains

constant ($k_2 = 318 \pm 4$ M^{-1} s^{-1}) between pH 7.0 and 9.0, and decreases to 273 M^{-1} s^{-1} at pH 10.0. This pH dependence reflects a complex mechanism that will be discussed in the next section. Data are means of four or five experiments, and the experimental errors in the k_2 values were less than ~7% in every case.

The second-order rate constants for the reaction of ascorbic acid 1 with 7-*tert*-butyl-5-isopropyltocopheroxy 2 have also been measured in an organic solvent system. A k_2 of 49 M^{-1} s^{-1} was obtained in benzene/ethanol (2:1, v/v) solution. The observed k_2 value is listed in Table I together with those observed in micellar solution.

DISCUSSION

The reduction of nitroxide free radicals by ascorbic acid 1 in solution has been studied by several investigators using EPR spectroscopy (13–16). A notable pH dependence of the second-order rate constant has been reported for the reaction between ascorbic acid 1 and nitroxide radical (4-hydroxy-2,2,6,6-tetramethylpiperidinyl-1-oxy radical) in micellar solution, suggesting that the reducing form of ascorbic acid is the ascorbate monoanion (AsH^-).

In this work, the rates of reaction of vitamin C (ascorbic acid 1) with the tocopheroxyl radical (7-*tert*-butyl-5-isopropyltocopheroxyl 2) in Triton X-100 micellar solutions have been determined spectrophotometrically using a stopped-flow technique. The observed second-order rate constants showed notable pH dependence with a broad maximum around pH 8.

Ascorbic acid 1 is dibasic and can exist in three different molecular forms, *i.e.* ascorbic acid (AsH_2), ascorbate monoanion (AsH^-) and ascorbate dianion (As^{2-}), depending on the pH value (see Fig. 1). The equilibrium reactions have the form:

$$AsH_2 \overset{K_{a1}}{\rightleftharpoons} AsH^- \overset{K_{a2}}{\rightleftharpoons} As^{2-} \qquad \text{(III)}$$

where $pK_{a1} = 4.17$ and $pK_{a2} = 11.57$ (17).

The graph of the pH dependence of k_2 (Fig. 5) has a peak at a pH value intermediate between the two pK_a values (pK_{a1} and pK_{a2}) of the dibasic ascorbic acid. Therefore, the mole fractions (f) present as the AsH_2 molecule and the AsH^- and As^{2-} ions were calculated as a function of pH. The analytical concentration (C_a) is given in Equation 2.

$$C_a = [AsH_2] + [AsH^-] + [As^{2-}] \qquad (2)$$

From the ionization constant expressions for ascorbic acid (AsH_2), we obtain the following.

$$[AsH^-] = \frac{[AsH_2]K_{a1}}{[H_3O^+]} \qquad (3)$$

$$[As^{2-}] = \frac{[AsH^-]K_{a2}}{[H_3O^+]} = \frac{[AsH_2]K_{a1}K_{a2}}{[H_3O^+]^2} \qquad (4)$$

Substitution into the expression for the analytical concentration (C_a) yields Equation 5.

$$f = \frac{[AsH_2]}{C_a} = \frac{[H_3O^+]^2}{[H_3O^+]^2 + [H_3O^+]K_{a1} + K_{a1}K_{a2}} \qquad (5)$$

Similarly, the expressions for the mole fractions present as AsH^- and As^{2-} can be derived from the following.

$$f = \frac{[AsH^-]}{C_a} = \frac{[H_3O^+]K_{a1}}{[H_3O^+]^2 + [H_3O^+]K_{a1} + K_{a1}K_{a2}} \qquad (6)$$

$$f = \frac{[As^{2-}]}{C_a} = \frac{K_{a1}K_{a2}}{[H_3O^+]^2 + [H_3O^+]K_{a1} + K_{a1}K_{a2}} \qquad (7)$$

Fractions of total ascorbic acid present as AsH_2, AsH^-, and As^{2-} are shown as functions of pH in Fig. 5.

As is clear from the results shown in Fig. 5, a good correlation between the rate constants (k_2) and mole fraction (f) of ascorbate (AsH^-) was observed. The result shows that the ascorbate monoanion (AsH^-) can regenerate tocopherol from tocopheroxyl in biological systems. In fact, as listed in Table I, sodium ascorbate (Na^+AsH^-) 3 and ascorbic acid 1 at pH 7.0 react with tocopheroxyl radical 2 at similar rates. Furthermore, the values of k_2 have been plotted against the mole fraction ($f = [AsH^-]/C_a$) of the ascorbate monoanion. As shown in Fig. 6, a good correlation between k_2 and $[AsH^-]/C_a$ was obtained. The results also indicate that ascorbic acid (AsH_2, the reduced form of ascorbic acid) does not have the ability to regenerate tocopherol from tocopheroxyl in aqueous solution.

In this work, we also measured the reaction rate (k_2) of ascorbic acid 1 with tocopheroxyl radical 2 in an organic solvent system. A rate constant of 49 M^{-1} s^{-1} was obtained in benzene/ethanol (2:1, v/v) solution. Ascorbic acid 1 is considered to exist in the reduced form (AsH_2) in organic solvent systems. Therefore, the results indicate that the reduced form of ascorbic acid (AsH_2) has the ability to regenerate tocopherol from tocopheroxyl in organic solvent systems. However, the former reaction rate ($k_2 = 49$ M^{-1} s^{-1}) in benzene/ethanol (2:1, v/v) solution is only ~15% of that ($k_2 = 322$ M^{-1} s^{-1}) in Triton X-100 micellar solution at pH 7.0.

As described above, Scarpa *et al.* (6) have measured the rate constant ($k_2 = 2 \times 10^5$ M^{-1} s^{-1}) for the reaction between the α-tocopheroxyl radical and ascorbic acid 1 in a liposomal system (pH 7.5, 22 °C). When comparing the above rate constant with that obtained in this work for the reaction of ascorbic acid 1 with 7-*tert*-butyl-5-isopropyltocopheroxyl 2 in Triton X-100 micellar solution (pH 7.0, 25 °C; $k_2 = 322$ M^{-1} s^{-1}), the former appears to be about 3 orders of magnitude higher than the latter. There are several factors that might influence the reaction rates, such as solvent, temperature, pH, the effect of steric hindrance of the alkyl groups at the *ortho*-positions of the attacking tocopheroxyl radical, etc. Since the pH values and temperatures were similar in each of these studies, the fourth factor (that is, the effect of steric hindrance) may be most important for the reaction between the tocopheroxyl radical and vitamin C. In this work, we measured the second-order rate constant for the reaction between aqueous vitamin C solutions and tocopheroxyl radical 2 in Triton X-100 micellar solution and found that the rate constant shows notable pH dependence. Therefore, similar pH dependences for the reaction between α-tocopheroxyl (vitamin E radical) and vitamin C may be expected.

The results of this study should provide a foundation for the interpretation of reactions between the tocopheroxyl radical and ascorbic acid in more complex biological systems.

REFERENCES

1. Mukai, K., Tsuzuki, N., Ouchi, S., and Fukuzawa, K. (1982) *Chem. Phys. Lipids* **30,** 337–345
2. Burton, G. W., Doba, T., Gabe, E. J., Hughes, L., Lee, F. L., Prasad, L., and Ingold, K. U. (1985) *J. Am. Chem. Soc.* **107,** 7053–7065
3. Tappel, A. L. (1968) *Geriatrics* **23,** 96–105
4. Packer, J. E., Slater, T. F., and Willson, R. L. (1979) *Nature* **278,** 737–738
5. Niki, E., Tsuchiya, J., Tanimura, R., and Kamiya, Y. (1982) *Chem. Lett.* 789–792
6. Scarpa, M., Rigo, A., Maiorino, M., Ursini, F., and Gregolin, C. (1984) *Biochim. Biophys. Acta* **801,** 215–219
7. Mukai, K., Fukuda, K., Ishizu, K., and Kitamura, Y. (1987) *Biochem. Biophys. Res. Commun.* **146,** 134–139
8. Mukai, K., Nishimura, M., Nagano, A., Tanaka, K., and Niki, E. (1989) *Biochim. Biophys. Acta* **993,** 168–173

9. Mukai, K., Kageyama, Y., Ishida, T., and Fukuda, K. (1989) *J. Org. Chem.* **54,** 552–556
10. Müller, E., Schick, A., and Scheffler, K. (1959) *Chem. Ber.* **92,** 474–482
11. Mukai, K., Watanabe, Y., and Ishizu, K. (1986) *Bull. Chem. Soc. Jpn.* **59,** 2899–2900
12. Mukai, K., Takamatsu, K., and Ishizu, K. (1984) *Bull. Chem. Soc. Jpn.* **57,** 3507–3510
13. Kocherginskii, N. M., S'yakste, N. I., Berkovich, M. A., and Devichenskii, V. M. (1981) *Biophysics* (*Engl. Transl. Biofizika*) **26,** 449–454
14. Craescu, C. T., Baracu, I., Grecu, N., Bucsa, L., and Niculescu-Duvaz, I. (1982) *Rev. Roum. Biochim.* **19,** 15–23
15. Liu, Y. C., Wu, L. M., Liu, Z. L., and Han, Z. X. (1985) *Acta Chim. Sin.* 342–348
16. Liu, Y. C., Liu, Z. L., and Han, Z. X. (1988) *Rev. Chem. Intermed.* **10,** 269–289
17. Windholz, M., Budavari, S., Blumetti, R. F., and Otterbein, E. S. (eds) (1983) *The Merck Index*, 10th Ed., p. 844, Merck & Co., Inc., Rahway, NJ