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Effects of solvents and media on the antioxidant activity of α-tocopherol

Misato Iwatsuki [a], Jyunichi Tsuchiya [b], Erika Komuro [b], Yorihiro Yamamoto [b], Etsuo Niki [a]

[a] *Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro, Tokyo 153, Japan,*
[b] *Department of Reaction Chemistry, Faculty of Engineering, The University of Tokyo, Hongo, Tokyo 113, Japan*

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Abstract

The effects of solvents and media on the antioxidant activity of α-tocopherol were studied. The antioxidant activities of α-tocopherol in different solvents decreased in the order of acetonitrile = hexane > ethanol = methanol, which indicates that the antioxidant activity of α-tocopherol is smaller in protic solvent than in aprotic solvent. The antioxidant activity of 2-(4,8,12-trimethyltridecyl)-5-hydroxy-2,4,6,7-tetramethylindan, which has similar structure to α-tocopherol but does not have ether oxygen, was also measured in protic and aprotic solvents. Its antioxidant activity was smaller than that of α-tocopherol in every solvent, but interestingly, substantially the same solvent effects were observed. These results show that the hydrogen bonding between the protic solvent and ether oxygen is not important but that the hydrogen bonding between protic solvent and phenolic group reduces the activity of α-tocopherol. Antioxidant activities of α-tocopherol in micelle system and liposomal membrane were markedly reduced compared with that in homogeneous solution. Solvent effect on the α-tocopheroxyl radical was also studied by using electron spin resonance. The hyperfine splitting constants of a_H^{5CH3} and a_H^{7CH3} were smaller in protic solvent than in aprotic solvent, which shows that lone-pair orbital energy on 5-CH3 and 7-CH3 is smaller in protic solvent. The ESR spectra of α-tocopheroxyl radical in liposomal membrane and micelle were similar to those observed in aprotic solvent and in protic solvent, respectively, suggesting that α-tocopheroxyl radical is located predominantly in the lipophilic domain of the liposomal membrane but in or closer to water phase of micelle aqueous suspensions.

Key words: α-Tocopherol; α-Tocopheroxyl radical; Antioxidant; Solvent effect; ESR; Lipid peroxidation; Liposomal membrane; SDS micelle

1. Introduction

There is now an increasing evidence which suggests the involvement of lipid peroxidation in a variety of pathological events, cancer and aging [1]. As a result, the function and action of various antioxidants have received much attention [2]. Vitamin E is now accepted as the major lipophilic, radical-scavenging antioxidant [3–5], which suppresses the oxidation of lipids by scavenging oxygen radicals to inhibit the chain initiation and/or break the chain propagation. The rate and mechanism of inhibition of oxidation by vitamin E, especially α-tocopherol having the highest bio-activity, has been studied extensively in homogeneous solution and now well understood [6,7]. On the other hand, the behavior of α-tocopherol in heterogeneous media, which are more relevant to biological system, has not been well elucidated yet [8]. It has been found that the antioxidant activity of α-tocopherol in the membrane is much smaller than that in homogeneous solution [9]. Furthermore, the rate constant for scavenging of lipid peroxyl radical by α-tocopherol has been found smaller in micelles [10,11] and in liposomal membranes [11,12] than in homogeneous solution. The reduced activity of α-tocopherol as an antioxidant against micelle and membrane oxidation in aqueous dispersions may be ascribed to lower mobility of α-tocopherol in aggregated medium [13] and/or hydrogen bonding by water at phenolic and/or chroman ether groups of α-tocopherol [10–12].

* Corresponding author. Fax: +81 (3) 34814574.

Abbreviations: PMC, 2,2,5,7,8-pentamethyl-6-chromanol; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; THMI, 2-(4,8,12-trimethyltridecyl)-5-hydroxy-2,4,6,7-tetramethylindan; 14: 0 PC, dimyristoyl phosphatidylcholine; ESR, electron spin resonance; DPPH, 2,2-diphenyl-1-picrylhydrazyl; AMVN, 2,2′-azobis (2,4-dimethylvaleronitrile); AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; SDS, sodium dodecyl sulfate.

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These observations suggest that the antioxidant potency of α-tocopherol in vivo depends much on the surrounding environment, for example, fluidity of the organized structures such as membranes and lipoproteins and also the presence of protic solvent such as water. Therefore, the present study was undertaken to investigate further the effects of solvent and medium on the antioxidant behavior of α-tocopherol. Methyl linoleate and soybean phosphatidylcholine were chosen as a substrate, since they give conjugated diene hydroperoxides almost quantitatively [14], which makes kinetic analysis for oxidation reliable, accurate and easy. Methanol and ethanol were used as a protic solvent and hexane and acetonitrile were chosen as an aprotic solvent. Micelles and liposomal membranes were used as a model for biological membranes. The effects of solvent and medium on the ESR spectrum of α-tocopheroxyl radical were also studied, since the behavior of α-tocopheroxyl radical is important in determining potency of α-tocopherol as an antioxidant.

2. Materials and methods

2.1. Materials

α-Tocopherol, trolox, PMC and THMI, whose structures are shown in Fig. 1, were kindly provided by Eisai Co. (Tokyo, Japan). Methyl linoleate was obtained from Tokyo Kasei Chemical Co. (Tokyo, Japan) and purified with silica-gel column before use. Commercial 14:0 PC obtained from Sigma Chemical Co. (St. Louis, MO) was used without further purification, while soybean PC obtained from Sigma Chemical Co. was purified by alumina and silica-gel columns [14]. A lipid-soluble azo compound, 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) and a water-soluble azo compound, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), used as radical sources, were obtained from Wako Pure Chemical Ind. (Osaka Japan). Solvents were also obtained from Wako Pure Chemical Ind. and treated with anhydrous sodium sulfate to remove water. DPPH was obtained from Aldrich Chemical Company (Milwaukee, WI). Sodium dodecyl sulfate (SDS) was purchased from Wako. The chemicals were of the highest grade commercially available.

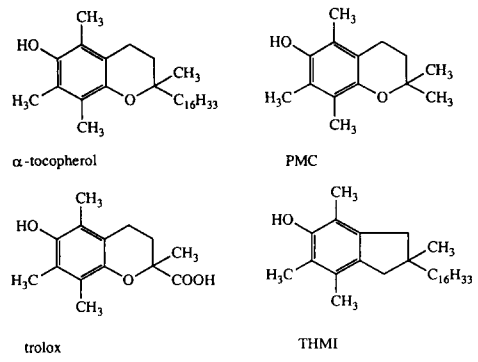

Fig. 1. Antioxidants used in this study.

2.2. Procedures

Oxidation procedures

Oxidations of methyl linoleate in homogeneous solutions. The oxidations of methyl linoleate were carried out in methanol, ethanol, acetonitrile and hexane in air at 37°C. The formation of methyl linoleate hydroperoxides was measured by following their conjugated diene with an HPLC equipped with a UV detector monitored at 234 nm. Samples were injected onto a reverse phase column (LC18; particle size 5 μm; 4.6 mm × 25 cm; Supelco, Tokyo) and methanol/H_2O (95/5 by volume) was used as an eluent with a flow rate of 1.0 ml/min. The retention time of methyl linoleate hydroperoxides was 4.6 min. When the oxidation was performed in hexane solution, methanol/*tert*-butyl alcohol/40 mM phosphate buffer (60/30/10 by volume) was used as an eluent and the retention time of the hydroperoxides was 6.0 min.

Oxidation of methyl linoleate micelle in SDS. The methyl linoleate micelles were prepared by mixing appropriate amounts of methyl linoleate, AMVN and α-tocopherol, when required, with an aqueous solution of 0.5 M SDS followed by vigorous mixing with a vortex mixer for 2 min. The rate of oxidation was measured by oxygen uptake using an oxygen monitor equipped with a Clark-type oxygen electrode (YSI Model 5300, Ohio). Oxidation was carried out at 37°C in air.

Oxidation of phosphatidylcholine liposomal membrane. The multilamellar liposomal membranes were prepared as follows as reported previously [14]. Appropriate amounts of soybean PC, AMVN, and α-tocopherol, when necessary, were dissolved in methanol and the solution was taken into a round bottle shaped flask. Methanol was removed under reduced pressure to obtain a thin film on the flask wall, which was slowly peeled off by shaking with 0.1 M NaCl aqueous solution containing 100 μM EDTA to obtain white milky aqueous suspensions of multilamellar liposomal membranes. The oxidation of PC liposomal membrane was carried out at 37°C in air and the accumulation of soybean PC hydroperoxides was measured by an HPLC using a UV detector at 234 nm. Straight phase column (LC-Si; particle size 5 μm; 4.6 mm × 25 cm; Supelco, Tokyo) was used and an eluent was methanol/40 mM phosphate buffer (90/10 by volume), flow rate being 1.0 ml/min. The retention time was 6.4 min.

In every medium, the antioxidant activity of α-tocopherol was evaluated from the induction period and the ratio of inhibited rate of oxidation to uninhibited oxidation, that is, how long and how much does α-tocopherol suppress the oxidation [6,7,9,15]. The oxidations were carried out repeatedly and the results were reproducible within ±10% of experimental error. The typical examples of the results are shown.

2.3. ESR study on α-chromanoxyl radical in various solvents and media

The ESR spectrum of α-chromanoxyl radical was measured in hexane, methanol, ethanol, acetonitrile and water. α-Chromanoxyl radical was formed by the interaction of α-tocopherol with DPPH or peroxyl radical produced by the decomposition of AMVN in various kinds of organic solvents. In water, trolox was used instead of α-tocopherol and AAPH was used as a radical source. In methanol, trolox, α-tocopherol and PMC were all used as sources of α-chromanoxyl radical and AMVN and AAPH were used as radical sources. It was confirmed that the identical ESR signal of α-chromanoxyl radical was obtained independent of the type of both the side chain of chromanol and the initiator.

The multilamellar liposomal membrane and SDS micelle were prepared as described above and the unilamellar liposomal membrane was prepared with a method using ethanol [16]. Appropriate amounts of 14:0 PC or soybean PC and antioxidants were dissolved in ethanol, which was slowly added into 0.1 M NaCl aqueous solution containing 100 μM EDTA while vigorously stirring. The volume of ethanol in the total suspensions was kept < 7%. AMVN and AAPH were used as radical sources. In the experiments using AAPH, an appropriate amount of AAPH was added as an aqueous solution to the unilamellar liposomal suspensions or SDS micelle suspensions to start the oxidation at 37°C under air. When AMVN was used as a radical initiator, it was incorporated into multilamellar liposomal membranes or micelles and they were incubated at 37°C in air.

Samples dissolved in polar solvents such as water and methanol were taken into a flat cell and ESR spectra were recorded on an X-band JEOL FE1X spectrometer. Samples dissolved in nonpolar solvents, like hexane or benzene, were measured in a capillary tube.

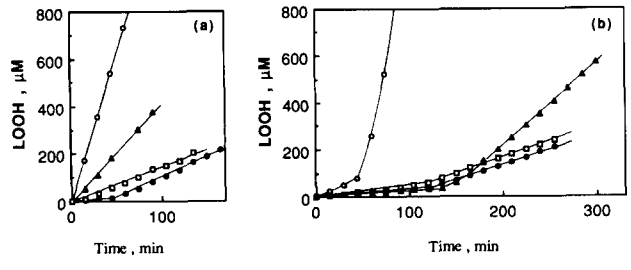

Fig. 2. Accumulation of methyl linoleate hydroperoxides (LOOH) during the oxidations of 453 mM methyl linoleate in the (a) absence and (b) presence of 2 μM α-tocopherol induced by 0.20 mM AMVN in acetonitrile (○), hexane(▲), methanol (□) and ethanol (●) at 37°C in air.

3. Results

3.1. Antioxidant activity of α-tocopherol against the oxidations of methyl linoleate in various solvents

Fig. 2a shows the results of oxidations of methyl linoleate induced by AMVN in the absence of antioxidant in four solvents: acetonitrile, hexane, methanol and ethanol. The oxidation of methyl linoleate induced by AMVN proceeds by a free radical chain mechanism and gives four kinds of conjugated diene hydroperoxides quantitatively as primary products [17–19]. Therefore, it is possible to measure the rate of oxidation quantitatively by following the amount of conjugated diene formed. As shown in Fig. 2, the oxidations of methyl linoleate proceeded at a constant rate without any noticeable induction period in methanol, hexane and acetonitrile. In ethanol, somewhat slower rate was observed initially, followed by constant steady oxidation. The initial lag may be ascribed to a contaminant such as thiophene. The rate of oxidation was fastest in acetonitrile, and decreased in the order of hexane, methanol and ethanol.

Fig. 2b. shows the formations of methyl linoleate hydroperoxides in various solvents in the presence of 2 μM α-tocopherol. α-Tocopherol markedly suppressed the oxidation in all of four kinds of solvents and produced a induction period after which a fast oxidation took place. The rates of oxidation after the induction period were similar to those in the absence of α-tocopherol.

Fig. 3 summarizes the lengths of the induction period produced by α-tocopherol in various solvents. It shows that the lengths of the induction period were proportional to the concentrations of α-tocopherol as observed previously [6,7,9,15]. α-Tocopherol produced shorter induction period in acetonitrile.

Fig. 4 shows the plot of the ratio of the rate of oxidation inhibited by α-tocopherol (R_{inh}) to the rate of oxidation in the absence of antioxidant (R_o) against a reciprocal of α-tocopherol concentration. This ratio (R_{inh}/R_o) gives the efficacy of inhibition of oxidation by an antioxidant. As shown in this figure, α-tocopherol showed a good antioxidant activity against the oxidations of methyl linoleate in hexane and in acetonitrile, but its antioxidant activity was smaller in ethanol and

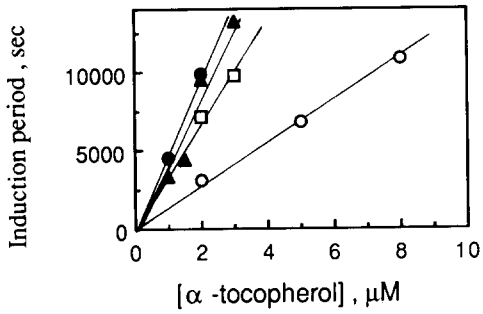

Fig. 3. Plot of the length of induction period as a function of α-tocopherol concentration in the oxidations of 453 mM methyl linoleate induced by 0.20 mM AMVN in acetonitrile (○), hexane (▲), methanol (□) and ethanol (●) at 37°C in air. The points in this figure are the averages of several experiments.

in methanol. Thus the antioxidant activity of α-tocopherol was smaller in protic solvent than in aprotic solvents, implying that this reduction in antioxidant activity of α-tocopherol may well be due to the hydrogen bonding by the solvent.

The contribution of a hydrogen bonding was supported by the ESR study which is described later in this paper. Two positions are possible for hydrogen bonding, phenolic group at the 6th position and ether oxygen. To clarify which is more important, the following experiments were carried out.

3.2. Antioxidant activities of α-tocopherol and THMI in methanol and hexane

Antioxidant activities of α-tocopherol and THMI, whose structure is similar to that of α-tocopherol but without chroman oxygen (see Fig. 1), have been measured in protic methanol and aprotic hexane. The examples of the results are shown in Fig. 5. THMI also suppressed the oxidation of methyl linoleate but its antioxidant activity was smaller than that of α-tocopherol. As shown in Fig. 5, the activities of both antioxidants were reduced markedly in methanol compared in hexane. The ratio R_{inh}/R_o in methanol was about 6 times larger than that in hexane for both α-tocopherol and THMI (Fig. 6), suggesting that the reduction in antioxidant activity of α-tocopherol in protic solvent is not because of the hydrogen bonding between chroman ether oxygen and protic solvent but due to the hydrogen bonding with the phenolic hydroxyl group.

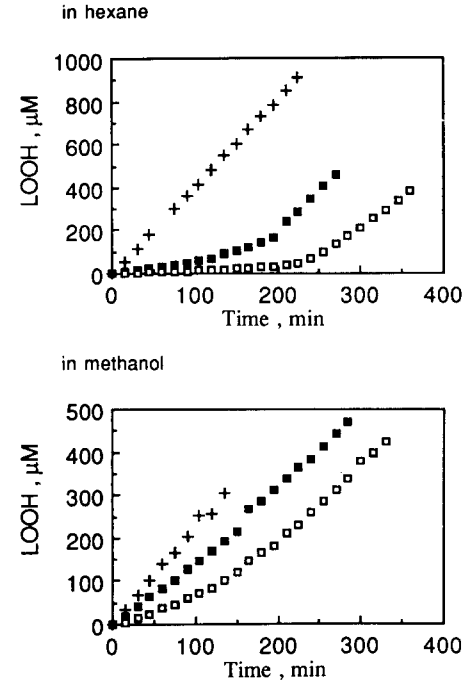

Fig. 5. Accumulation of methyl linoleate hydroperoxides (LOOH) during the oxidations of 453 mM methyl linoleate induced by 0.20 mM AMVN in the absence of antioxidant (+) and presence of either 3 μM α-tocopherol (□) or 3 μM THMI (■) in hexane (upper figure) and in methanol (lower figure) at 37°C in air. The data shown is a typical example from several independent experiments.

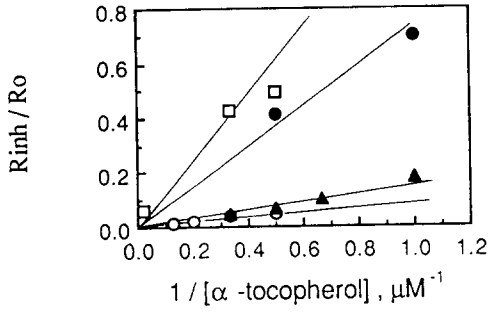

Fig. 4. Plot of the ratio of the rate of oxidation inhibited by α-tocopherol to that of non-inhibited oxidation (R_{inh}/R_o) as a function of the reciprocal of α-tocopherol concentration in the oxidation of 453 mM methyl linoleate induced by 0.20 mM AMVN in acetonitrile (○), hexane (▲), methanol (□) and ethanol (●) at 37°C in air. The points in this figure are the averages of several experiments.

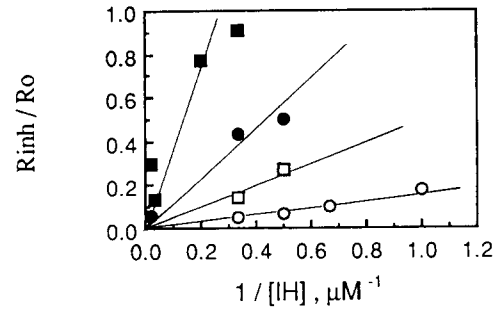

Fig. 6. Plot of the ratio of the rate of oxidation inhibited by either α-tocopherol (circle) or THMI (square) to that of non-inhibited oxidation (R_{inh}/R_o) as a function of a reciprocal of antioxidant concentration (1/[IH]) in the oxidation of 453 mM methyl linoleate induced by 0.20 mM AMVN in methanol (closed mark) and in hexane (open mark) at 37°C in air. The points are the averages of several experiments.

3.3. Antioxidant activities of α-tocopherol in SDS micelle or phosphatidylcholine liposomal membrane

The oxidations of methyl linoleate micelle and soybean PC liposomal membrane induced by AMVN proceed by a free radical chain mechanism. The examples of the oxidations of methyl linoleate micelle and soybean PC liposomal membrane in the absence and presence of α-tocopherol are summarized in Table 1. These data and previous reports [9,12] show that α-tocopherol suppressed the oxidation of lipids in both media efficiently. The lengths of the induction period by α-tocopherol were directly proportional to the concentration of α-tocopherol in both micelle and liposomal systems and the rates of inhibited oxidation (Rinh) were inversely proportional to the concentration of α-tocopherol (data not shown).

3.4. ESR study on α-tocopheroxyl radical in different solvents and media

An ESR signal of α-tocopheroxyl radical has been reported [20–24] and its hyperfine splitting constants have been measured. In this study, ESR signals of α-tocopheroxyl radicals were measured in various solvents. Fig. 7a shows the ESR spectrum of α-tocopheroxyl radical in benzene under vacuum. As shown in this figure, the hyperfine signal of α-tocopheroxyl radical could be observed in nonpolar solvent and in the absence of oxygen and the hyperfine

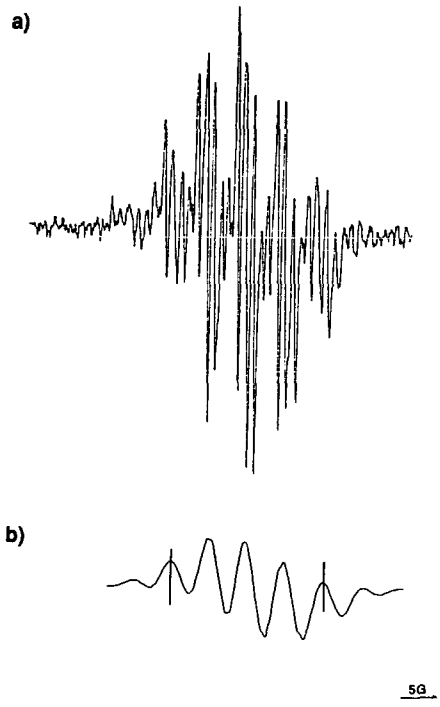

Fig. 7. ESR spectra of α-tocopheroxyl radical observed in benzene when α-tocopherol was mixed with DPPH (3/1 by mol/mol) (a) under vacuum and (b) in air.

splitting constants were obtained as $a_H^{5CH3} = 0.51$ mT, $a_H^{7CH3} = 0.45$ mT, $a_H^{4CH3} = 0.15$ mT, and $a_H^{8CH3} = 0.10$ mT, which are in good agreement with the data re-

Table 1
Hyperfine coupling constant for α-tocopheroxyl radical and kinetic parameters for the oxidations of methyl linoleate and soybean PC liposomes in different solvents and media

	Homogeneous solution					Micelle [a]	Liposome [a]
	H_2O	MeOH	EtOH	CH_3CN	Hexane		
Coupling constant, mT [b]	0.45	0.47	0.50	0.51	0.51	0.48	0.51
[LH], M		0.453	0.453	0.453	0.453	0.143 [2860]	0.0051 [1158]
[AMVN], mM		0.20	0.20	0.20	0.20	5.0 [100]	1.0 [227]
[α-toc], μM		2.0	2.0	2.0	2.0	2.0 [40]	2.0 [454]
10^{-3} $t$inh, s [c]		7.16	9.90	3.12	9.54	4.80	7.08
Ri, nM/s [d]		0.559	0.404	1.28	0.419	8.33 [167]	0.565 [128]
Ro, nM/s [e]		18.9	20.7	158	67.2	116 [2324]	35.2 [7992]
(kcl)o [f]		33.8	51.2	123	160	13.9	62.3
Rinh, nM/s [g]		9.37	8.55	9.48	4.77	80.8 [1616]	2.23 [507]
(kcl)inh [h]		16.8	21.2	7.41	11.4	9.7	3.95
Rinh/Ro		0.50	0.41	0.060	0.071	0.70	0.063
10^{-3} $k$inh/kp [i]		6.8	5.4	15	10	3.7	0.32

[a] Numbers in the brackets are for lipid compartment, not for whole aqueous suspensions.
[b] Hyperfine coupling constant for radicals from Trolox in water and from α-tocopherol in other solvents at 37°C, experimental error, < ±5%.
[c] Induction period.
[d] Rate of chain initiation calculated from Ri = 2 [7α-tocopherol]/$t$inh [6,7].
[e] Rate of oxidation in the absence of α-tocopherol.
[f] Kinetic chain length without α-tocopherol, Ro/Ri.
[g] Rate of oxidation inhibited by α-tocopherol.
[h] Kinetic chain length in the presence of α-tocopherol, Rinh/Ri.
[i] Caluculated from $k$inh/kp = [LH]/$t$inh Rinh [25], $k$inh and kp are the rate constants for scavenging of peroxyl radical by α-tocopherol and chain propagation respectively.

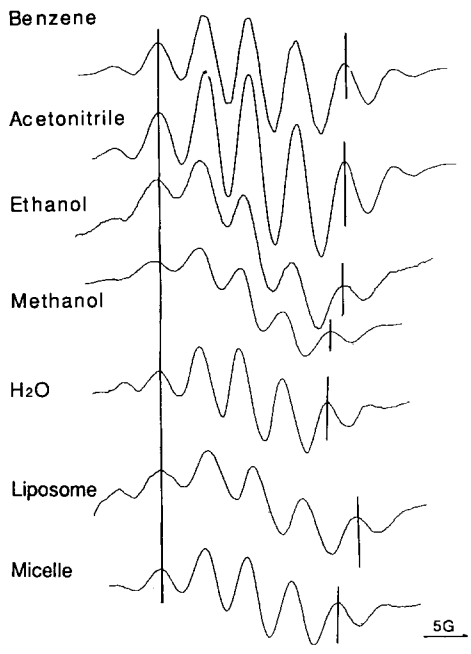

Fig. 8. ESR spectrum of α-tocopheroxyl radical in various solvents and media. In benzene, acetonitrile, methanol and ethanol, α-tocopherol was treated with DPPH. In water, trolox was used instead of α-tocopherol and AAPH was used as a radical source. In liposomes, 5.0 mM α-tocopherol was incorporated into 59 mM 14:0 PC liposomal membrane and 200 mM AAPH was used as an initiator. In micelle, 10 mM α-tocopherol and 52 mM methyl linoleate were dispersed in 0.5 M SDS and 200 mM AAPH was used as an initiator. Samples dissolved in hexane or acetonitrile were taken into capillary tube and measured under reduced pressure (approximately 0.5 atm. of air) at 37°C. Others were carried out at 37°C and measured in air.

ported previously [23,24]. Fig. 7b shows the ESR spectrum of α-tocopheroxyl radical in the presence of air. Only the spectrum of 7 lines was obtained. The hyperfine splitting constants of 7 lines are the average of those of a_H^{5CH3} and a_H^{7CH3}, which depend on the lone-pair orbital energy on H^{5CH3} and H^{7CH3}.

Fig. 8 shows the ESR signals of α-tocopheroxyl radical in various solvents. The experimental conditions were chosen to obtain similar signal intensities in different media. The average splitting constants of a_H^{5CH3} and a_H^{7CH3} are summarized in Table 1. They were larger in aprotic solvents than in protic solvents.

The ESR spectra of α-tocopheroxyl radicals were also measured in liposomal membrane and micelle. Soybean PC or 14: 0 PC was used to prepare liposomal membrane and AMVN or AAPH was used as radical source. The same ESR spectra were observed from α-tocopherol in the liposomes independent of phospholipid and radical source and their hyperfine splitting constants were similar to those obtained in hexane and acetonitrile (Fig. 8 and Table 1). The same spectrum was obtained from PMC as that of α-tocopherol as observed previously [25]. When α-tocopherol was incorporated into SDS micelles, the ESR spectrum of α-tocopheroxyl radical was similar to that in water or methanol (Fig. 8 and Table 1).

4. Discussion

As shown above, the solvents and media affected the rate of oxidation of methyl linoleate, antioxidant behavior of α-tocopherol, and ESR spectrum of α-tocopheroxyl radical.

Fig. 3 shows that the induction period was directly proportional to the antioxidant concentration and Fig. 4 shows that the ratio of the rate of oxidation inhibited by α-tocopherol to that in the absence of antioxidant was inversely proportional to the concentration of α-tocopherol, as observed previously [25]. These results indicate that oxidation inhibited by α-tocopherol proceeds by a conventional free radical chain mechanism and follows the classical rate law of autoxidation [6,7].

In addition to the coupling constants for α-tocopheroxyl radical in various media, the kinetic parameters are summarized in Table 1 for the oxidations of methyl linoleate and soybean PC in various media. Fig. 3 and Table 1 show that the rate of chain initiation by AMVN depends on solvent. It is larger in acetonitrile than in hexane, ethanol, or methanol. The rate of oxidation in the absence of α-tocopherol (R_o) was also fastest in acetonitrile (Fig. 2 (a) and Table 1), but the difference in the rate of chain initiation does not fully account for the difference in the rate of oxidation. This may be ascribed, at least in part, to the high polarity of acetonitrile, since the oxidizability of the hydrocarbons is known to be increased with increasing polarity of the solvent [26]. The rate constatnt for unimolecular decomposition and efficiency of chain initiation of an azo compound are relatively insensitive to solvent polarity [26]. The apparent high rate of chain initiation observed in acetonitrile may well be due to a less efficient scavenging of peroxyl radicals by α-tocopherol in acetonitrile, since the net mumber of peroxyl radicals scavenged by one molecule of α-tocopherol is smaller than 2 [8,27]. Table 1 shows that the kinetic chain length in the absence and presence of α-tocopherol was always larger than 1, suggesting that the oxidations proceeded by a free radical chain mechanism.

Table 1 also shows the antioxidant efficacy of α-tocopherol in different solvents and media. The ratio, R_{inh}/R_o, gives the extent of inhibition of oxidation by an antioxidant and the ratio k_{inh}/k_p gives the activity of antioxidant, where k_{inh} and k_p are the rate constants for scavenging peroxyl radical by α-tocopherol and chain propagation respectively. Table 1 indicates that the antioxidant activity of α-tocopherol is smaller in protic alcohol solution than in aprotic solution. Considering the high concentration of α-tocopherol in

lipophilic compartment of micelle and liposome, that is, the high α-tocopherol/lipid ratio, it can be concluded that the antioxidant activity of α-tocopherol is much smaller in micelle and liposomal membrane as observed previously [9].

A smaller antioxidant potency of α-tocopherol in protic solvent implies the hydrogen bonding between solvent and α-tocopherol. Fig. 5 shows that the antioxidant activities of α-tocopherol and THMI were markedly smaller in methanol than in hexane. Fig. 6 shows that the ratio R_{inh}/R_o was about 6 times larger in methanol than in hexane for both α-tocopherol and THMI, suggesting that these antioxidants suppressed the oxidation of methyl linoleate 6 times more efficiently in hexane than in methanol. Barclay et al. [12] also found that the inhibition rate constant k_{inh} for α-tocopherol in aprotic chlorobenzene was larger than that in protic *tert*-butyl alcohol. Pryor et al. [10] also reported that the rate constant k_{inh} was smaller in the aqueous system. They interpreted the smaller rate constant k_{inh} in protic solvent than in aprotic solvents by the hydrogen bonding between protic solvent and both the ether and phenolic oxygens of α-tocopherol [10,12]. However, the results observed in the present study that methanol reduced the antioxidant activities of both α-tocopherol and THMI to the same extent independent of the presence or absence of chromanoxyl ether oxygen suggest that the hydrogen bonding by protic solvent with ether oxygen of α-tocopherol does not play an important role in modulating its antioxidant activity.

Fig. 8 and Table 1 show that the solvents also interact with the α-tocopheroxyl radical. In protic solvent, the hyperfine splitting constants of a_H^{5CH3} and a_H^{7CH3} were smaller than those in aprotic solvent. These results can be interpreted by the hydrogen bonding between solvent and α-tocopheroxyl radical. That is, such hydrogen bonding increases the lone-pair orbital energy on oxygen atom of α-tocopheroxyl radical, which lower the lone-pair orbital energy on H^{5CH3} and H^{7CH3} and the hyperfine splitting constants of a_H^{5CH3} and a_H^{7CH3} [28–31].

The position of α-tocopherol in the membrane has been the subject of extensive studies and debate [32–38]. It is now accepted that α-tocopherol is retained in the membrane in such a way that the hydroxyl group is placed at or near the surface and the phytyl side chain is embedded into the membrane. However, there has been little study on the position of α-tocopheroxyl radical in the membrane. The data in Fig. 8 and Table 1 show that the coupling constants of a_H^{5CH3} and a_H^{7CH3} of α-tocopheroxyl radical in the membrane are similar to those in aprotic solvents, indicating that α-tocopheroxyl radical does not stick its phenolic group out of the membrane into the aqueous phase but rather situated in the lipophilic domain of the membrane. But, of course, it should be close to the surface of the membrane since it is reduced by ascorbate [39,40] and this reduction becomes less efficient as the radical goes deeper into the interior of the membrane [41]. On the other hand, phenolic oxygen of α-tocopheroxyl radical in the micelle is suggested from the data in Fig. 8 and Table 1 to be located in or closer to water phase, which is in agreement with the result reported by Simic [42].

The contributuion of α-tocopherol as a prooxidant has been proposed recently [43–45]. That is, α-tocopherol which resides at or near the surface of membrane reacts more rapidly with attacking aqueous radicals than the lipid to give α-tocopheroxyl radical, which attacks lipid to give lipid radical to initiate the chain reaction. Thus, α-tocopherol is assumed to behave as a chain-carrier and enhance the efficiency of lipid radical formation. The hydrogen atom abstraction from polyunsaturated fatty acids by chromanoxyl radicals has been observed in solution [46]. The overall potency of α-tocopherol as an antioxidant depends on the fate of α-tocopheroxyl, but it is not clear at present how such a prooxidant action of α-tocopherol is important in the membranes and lipoprotein in vivo where the vertical motion of α-tocopherol is restricted and where the reductants may well react with α-tocopheroxyl radical to regenerate α-tocopherol [2,8,47].

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References

[1] Sies, H. (Ed.) (1991) Oxidative Stress: Oxidants and Antioxidants. Academic Press, London.
[2] Niki, E., Noguchi, N. and Gotoh, N. (1993) Biochem. Soc. Trans. 21, 313–317.
[3] Burton, G.W., Joyce, C. and Ingold, K.U. (1983) Arch. Biochem. Biophys. 221, 281–290.
[4] Machlin, L. in Handbook of Vitamins (Machlin, L., Ed.,) Marcel Dekker, New York (1991) pp. 99–144.
[5] Packer, L. and Fuchs, J., eds. (1992) Vitamin E in Health and Disease, Marcel Dekker, New York.
[6] Burton, G.W. and Ingold, K.U. (1986) Acc. Chem. Res. 19, 194–201.
[7] Niki, E. (1987) Chem. Phys. Lipids 44, 227–253.
[8] Burton, G.W., Hughes, L., Foster, D.O., Pietrzak, E., Goss-Sampson, M.A. amd Muller, D.P.R. (1993) in Free Radicals: From Science to Medicine (Poli, G., Albano, E. and Dianzani, M.U., eds.) Birkhauser Verlag, Basel, pp. 388–399.
[9] Niki, E., Takahashi, M. and Komuro, E. (1986) Chem. Lett., 1573–1576.
[10] Pryor, W.A., Strickland, T. and Church, D.F. (1988) J. Am. Chem. Soc. 110, 2224–2229.
[11] Barclay, L.R.C., Baskin, K.A., Locke, S.J. and Vinguist, M.B. (1989) Can. J. Chem. 67, 1366–1369.

[12] Barclay, L.R.C., Baskin, K.A., Dakin, K.A., Locke, S.J. and Vinguist, M.B. (1990) Can. J. Chem. 68, 2258–2269.
[13] Gotoh, N., Shimizu, K., Komuro, E., Tsuchiya, J., Noguchi, N. and Niki, E. (1992) Biochim. Biophys. Acta. 1128, 147–154.
[14] Yamamoto, Y., Niki, E., Kamiya, Y. and Shimasaki, H. (1984) Biochim. Biophys. Acta. 795, 332–340.
[15] Barclay, L.R.C., Baskin, K.A., Kong, D. and Locke, S.J. (1987) Can. J. Chem., 65, 2541–2550.
[16] Batzri, S. and Korn, E.D. (1973) Biochim. Biophys. Acta. 298, 1015–1019.
[17] Porter, N.A. (1986) Acc. Chem. Res. 19, 262–268.
[18] Yamamoto, Y., Niki, E. and Kamiya, Y. (1982) Bull. Chem. Soc. Jpn. 55, 1548–1550.
[19] Yamamoto, Y., Niki, E. and Kamiya, Y. (1982) Lipids 17, 870–877.
[20] Kohl, D.H., Wright, J.R. and Weissman, M. (1969) Biochim. Biophys. Acta. 180, 536–544.
[21] Boguth, W. and Niemann, H. (1971) Biochim. Biophys. Acta. 248, 121–130.
[22] Ozawa, T., Hanaki, A., Matsumoto, S. and Matsuo, M. (1978) Biochim. Biophys. Acta. 531, 72–78.
[23] Mukai, K., Tsuzuki, N., Ishizu, K., Ouchi, S. and Fukuzawa, K. (1981) Chem. Phys. Lipids 29, 129–135.
[24] Niki, E., Tsuchiya, J., Tanimura, R. and Kamiya, Y. (1982) Chem. Lett. 789–792.
[25] Niki, E., Kawakami, A., Saito, M., Yamamoto, Y., Tsuchiya, J., Kamiya, Y. (1985) J. Biol. Chem. 260, 2191–2196.
[26] Niki, E., Kamiya, Y. and Ohta, N. (1969) Bull. Chem. Soc. Jpn. 42, 3224–3229.
[27] Liebler, D.L., Baker, P.F. and Kaysen, K.L. (1990) J. Am. Chem. Soc. 112, 6995–7000.
[28] Stone, E.W. and Maki, A.H. (1962) J. Chem. Phys. 36, 1944–1945.
[29] Piette, L.H., Ludwig, P. and Adams, R.N. (1962) J. Am. Chem. Soc. 84, 4212–4215.
[30] Deguchi, Y. (1962) Bull. Chem. Soc. Jpn. 35, 260–264.
[31] Umemoto, K., Deguchi, Y. and Takaki, H. (1963) Bull. Chem. Soc. Jpn. 36, 560–563
[32] Takahashi, M., Tsuchiya, J. and Niki, E. (1989) J. Am. Chem. Soc. 111, 6350–6353.
[33] Kagan, V.E. and Quinn, P.J. (1988) Eur. J. Biochem. 171, 661–667.
[34] Perly, B., Smith, I.C.P., Hughes, L., Burton, G.W. and Ingold, K.U. (1985) Biochim. Biophys. Acta 819, 131–135.
[35] Urano, S., Iida, M., Otani, I. and Matsuo, M. (1987) Biochem. Biophys. Res. Commun. 146, 1413–1418.
[36] Wassall, S.R., Thewalt, J.L., Wong, L., Gorrissen, H. and Cushley, R.J. (1986) Biochemistry. 25, 319–326.
[37] Ekiel, I.H. Hughes, L., Burton, G.W., Jovall, P.A., Ingold, K.U. and Smith, I.C.P. (1988) Biochemistry. 27, 1432–1440.
[38] Kagan, V.E., Serbinova, E.A., Bakalova, R.A., Novikov, K.V., Skrypin, V.I. Evstigneeva, R.P. and Stoychev, Ts. S. (1987) Free Radicals, Oxidant Stress and Drug Action. London, Richelieu Press, 425–442.
[39] Niki, E., Kawakami, A., Yamamoto, Y., and Kamiya, Y. (1985) Bull. Chem. Soc. Jpn. 58, 1971–1975.
[40] Doba, T., Burton, G., W., and Ingold, K.U. (1985) Biochim. Biophys. Acta 835, 298–303.
[41] Takahashi, M., Tsuchiya, J., Niki, E., and Urano, S. (1988) J. Nutr. Sci. Vitaminol. 34, 25–34.
[42] Simic, M.G. (1981) Oxygen and Oxy-Radicals in Chemistry and Biology, pp. 109–118, Academic Press, New York.
[43] Bowry, V.W., Ingold, K.U. and Stocker, R. (1992) Biochem. J. 288, 341–344.
[44] Ingold, K.U., Bowry, V.W., Stocker, R. and Walling, C. (1993) Proc. Natl. Acad. Sci. USA 90, 45–49.
[45] Bowry, V.W. and Stocker, R. (1993) J. Am. Chem. Soc. 115, 6029–6044.
[46] Mukai, K., Morimoto, H., Okauchi, Y. and Nagaoka, S. (1993) Lipids 28, 753–756, and references cited therein.
[47] Niki, E. (1987) Ann. N.Y. Acad. Sci. 498, 186–199.