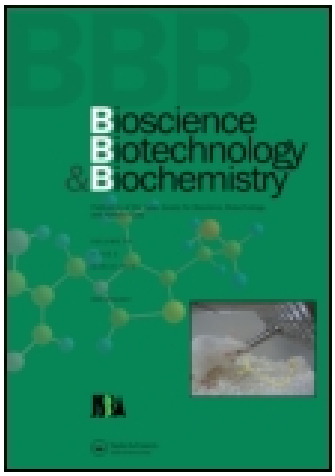

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Oxidation of α-Tocopherol during the Peroxidation of Dilinoleoylphosphatidylcholine in Liposomes

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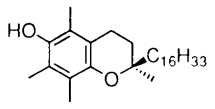
Liposomal suspensions of dilinoleoylphosphatidylcholine (DLPC) containing α-tocopherol (0.1 mol%, based on DLPC) were oxidized at 37°C. The oxidation was initiated by a lipid-soluble or water-soluble free radical initiator, or by the addition of $CuSO_4$ and fructose. In all the oxidation systems, α-tocopherol suppressed the formation of DLPC hydroperoxides until all the α-tocopherol had been depleted. The oxidation products of α-tocopherol were 8a-alkyldioxy-α-tocopherones, 5,6-epoxy-α-tocopherylquinone, 2,3-epoxy-α-tocopherylquinone, and α-tocopherylquinone. The 8a-alkyldioxy-α-tocopherones were decomposed in the liposomes primarily by being hydrolyzed to produce α-tocopherylquinone. The results indicate that α-tocopherol can trap peroxyl radicals to form 8a-alkyldioxy-α-tocopherones which are hydrolyzed to α-tocopherylquinone in phospholipid bilayers. In another oxidation pathway, α-tocopherol may be oxidized by peroxyl radicals to form isomeric epoxy-α-tocopherylquinones.

Key words: α-tocopherol; vitamin E; lipid peroxidation; liposomes

α-Tocopherol (vitamin E, **1**) is generally considered to protect biological membranes from lipid peroxidation.[1–3] The antioxidative activity of α-tocopherol is related to scavenging the peroxyl radicals of unsaturated lipids in the membranes. α-Tocopherol efficiently transfers a hydrogen atom to a peroxyl radical, giving a hydroperoxide and an α-tocopheroxyl radical (**2**).[1] The α-tocopheroxyl radical, once formed, reacts with a second peroxyl radical to form a non-radical product. Thus, each molecule of α-tocopherol that is consumed traps two peroxyl radicals. The reaction products of α-tocopherol with peroxyl radicals have been extensively studied to explain the mechanism for autoxidation inhibition by α-tocopherol.[4–15] The reaction of peroxyl radicals with the α-tocopheroxyl radical forms 8a-substituted α-tocopherones, and often involves the coupling of alkylperoxyl radicals with the tocopheroxyl radical to form 8a-alkyldioxy-α-tocopherones (**3**).[4–11,14,15]



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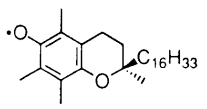

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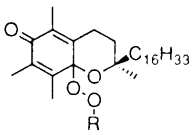
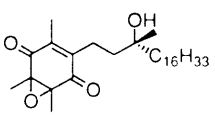


3a, R = phosphatidylcholine

3b, R = $C(CN)(CH_3)CH_2CH(CH_3)_2$



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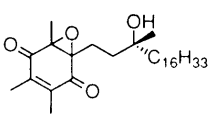

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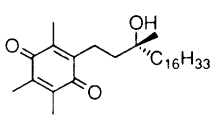

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Phospholipids are important structural components in biological membranes, and they are targets of lipid peroxidation. Phospholipid liposomal systems have been employed to model the peroxyl radical-trapping reactions of α-tocopherol in biological membranes.[11,12,15] We have previously reported the isolation and characterization of 8a-(phospholipid-dioxy)-α-tocopherones (**3a**) as primary products of the α-tocopheroxyl radical with phospholipid-peroxyl radicals during the autoxidation of phosphatidylcholine in liposomes.[15] The 8a-(phospholipid-dioxy)-α-tocopherones in the liposomal system accounted for only about 30 mol% of the consumed α-tocopherol, the rest being unknown products. These unknown products may involve isomeric epoxy-8a-hydroperoxy-α-tocopherones, their hydrolysis products, epoxy-α-tocopherylquinones (**4** and **5**), and α-tocopherylquinone (**6**).[11,12] However, the formation and the fate of these oxidation products in a liposomal system are still uncertain.

This paper reports the formation of oxidation products of α-tocopherol, including 8a-(phospholipid-dioxy)-α-tocopherones, during the peroxidation of phosphatidylcholine in two liposomal membrane systems. The liposomes used were multilamellar or large unilamellar vesicles of 1,2-dilinoleoyl-3-*sn*-phosphatidylcholine (DLPC). Peroxidation of the DLPC liposomes was started in the lipid phase by lipid-soluble 2,2′-azo*bis*(2,4-dimethylvaleronitrile) (AMVN) for the multilamellar liposomal system, and started in the aqueous phase by water-soluble 2,2′-azo*bis*(2-amidinopropane) dihydrochloride (AAPH) or a copper ion for the unilamellar liposomal system. The ratio of α-tocopherol to polyunsaturated fatty acids in most biological membranes is on the order of 1 : 1000,[16,17] so we

Abbreviations: AAPH, 2,2′-azo*bis*(2-amidinopropane) dihydrochloride; AMVN, 2,2′-azo*bis*(2,4-dimethylvaleronitrile); DLPC, 1,2-dilinoleoyl-3-*sn*-phosphatidylcholine; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; HPLC, high-performance liquid chromatography; PC-OOH, 1,2-dilinoleoyl-3-*sn*-phosphatidylcholine monohydroperoxides.

have chosen the concentration of α-tocopherol in DLPC liposomes to be 0.1 mol% relative to the DLPC concentration.

Materials and Methods

Materials. *RRR*-α-Tocopherol (type V) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and purified by reversed-phase high-performance liquid chromatography (HPLC).[15] *RRR*-γ-Tocopherol, which was used as an internal standard, was prepared from mixed isomers of tocopherol (Hohnen Oil Co.; Tokyo, Japan). The 8a-(phospholipid-dioxy)-α-tocopherones (**3a**) were prepared by the reaction of α-tocopherol with DLPC initiated by AMVN, and purified by HPLC as described peviously.[15] The AMVN-derived 8a-alkyldioxy-α-tocopherones (**3b**) were prepared by the reaction of α-tocopherol with AMVN in benzene.[18] The epoxy-α-tocopherylquinones (**4** and **5**) were obtained as oxidation products of α-tocopherol during the peroxidation of methyl linoleate in an aqueous dispersion.[19] α-Tocopherylquinone (**6**) was the oxidation product of α-tocopherol with $FeCl_3$ in ethanol.[20] The α-tocopherol dimer was synthesized by following the procedure of Nelan and Robeson.[21] DLPC was synthesized by 1,2-diacylation of L-α-glycerophosphocholine cadmium complex (Sigma) with linoleic anhydride according to the method of Gupta *et al.*,[22] and was purified by reversed-phase HPLC, using an Inertsil Prep-ODS column (1.0 × 20 cm, GL Sciences; Tokyo, Japan) with methanol to remove the contaminating hydroperoxides. Authentic DLPC monohydroperoxides (PC-OOH) were prepared by the oxidation of DLPC liposomes in the presence of 1 mol% α-tocopherol initiated by AMVN,[23] and purified by HPLC under the same condition as those already described. 1,2-Dimyristoyl-3-*sn*-phosphatidylcholine (DMPC) was purchased from Sigma, while the free-radical initiators, AMVN and AAPH, were obtained from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification.

Liposome preparation and incubation. Multilamellar liposomes of DLPC (10 mM) containing α-tocopherol (0.1 mol%, based on DLPC) and AMVN (20 mol%, based on DLPC) were prepared by coevaporating from ethanol and then vigorously mixing in a 50 mM sodium phosphate buffer (pH 7.4, containing 50 mM NaCl) as described previously,[15] and incubated at 37 °C under air with mechanical shaking. In this case, the liposomes were quickly prepared at 4 °C in order to prevent the start of AMVN decomposition. The liposomes containing 8a-(phospholipid-dioxy)-α-tocopherones (**3a**: 0.5 mol%, based on DLPC) and with or without AMVN (20 mol%, based on DLPC) were also prepared as just described, and incubated at 37 °C.

Large unilamellar liposomes containing α-tocopherol were prepared by the extrusion method.[24] DLPC containing α-tocopherol (0.1 mol%, based on DLPC) was suspended by vigorously mixing for 2 min in a 50 mM sodium phosphate buffer (pH 7.4, containing 50 mM NaCl) and then by sonicating for 2 min. The milky suspension was transferred into LiposoFast apparatus (Avestin, Ottawa, Canada), extruded 19 times back and forth through a polycarbonate membrane (100 nm pore size), and diluted with the same buffer to give a final DLPC concentration of 10 mM. After preincubating at 37 °C for 5 min, either water-soluble AAPH (final concentration of 2 mM) or a mixture of $CuSO_4$ and fructose (final concentrations of 10 μM and 10 mM, respectively) was added to the liposomes, and the mixture incubated at 37 °C under air. We did not investigate the AMVN-initiated peroxidation of unilamellar liposomes, because AMVN in the lipid phase decomposed during the liposome preparation.[25]

Product analysis. Each incubated mixture was extracted twice with two volumes of hexane–2-propanol (3 : 2, v/v),[11] and the extracts evaporated to dryness *in vacuo*. The lipid residue was dissolved in ethanol and analyzed by reversed-phase HPLC. The amount PC-OOH was determined by using a TSK-gel Octyl-80TS column (4.6 × 150 mm, Tosoh Co.; Tokyo, Japan) with methanol–water (95 : 5, v/v) at 1.0 ml/min. Peaks were monitored by the absorbance at 235 nm, α-tocopherol being quantified by HPLC with fluorescence detection.[15] The amount of 8a-(phospholipid-dioxy)-α-tocopherones (**3a**) was determined by using an Inertsil C8 column (4.6 × 150 mm) with methanol–water (99 : 1, v/v) at 1.0 ml/min and monitoring for absorbance at 240 nm.

To analyze the other oxidation products of α-tocopherol (**3b**, **4**–**6**), DLPC and its oxidation products, which coeluted with the oxidaton products of α-tocopherol, were removed by solid-phase extraction before the HPLC analysis. Each lipid extract was dissolved in 0.5 ml of hexane–ethyl acetate (1 : 1, v/v), and applied to a Sep-Pak silica cartridge (Waters, Milford, MA, U.S.A.) that had been prewetted with the same solvent. The oxidation products of α-tocopherol were eluted with 4 ml of the same solvent. The eluted fractions were pooled and evaporated under N_2. The residue was dissolved in ethanol and analyzed by reversed-phase HPLC with a Puresil 5 μ C18 120 Å column (4.6 × 150 mm, Nihon Waters; Tokyo, Japan) using methanol–acetonitrile–water (87 : 10 : 3, v/v) at 1.0 ml/min. Absorbance was monitored at 285 nm and γ-tocopherol was used as an internal standard.

Results

Reaction of α-tocopherol during the peroxidation of DLPC in multilamellar liposomes

DLPC liposomes containing 0.1 mol% α-tocopherol were reacted with peroxyl radicals generated in the lipid phase by the thermal decomposition of AMVN,[3] and the oxidation products of α-tocopherol were analyzed by reversed-phase HPLC (Fig. 1). The sample extracts in ethanol were directly injected into a C8 column, and the peaks corresponding to a mixture of isomeric DLPC-derived 8a-alkyldioxy-α-tocopherones (**3a**), which were the addition products of α-tocopherol with DLPC-peroxyl radicals,[15] appeared in the 3-h sample (Fig. 1A). The samples, after treatment with the solid-phase extraction, were injected into a C18 column (Fig. 1B). Along with unreacted α-tocopherol (**1**) and the internal standard (γ-tocopherol), peaks corresponding to 5,6-epoxy-α-tocopherylquinone (**4**), 2,3-epoxy-α-tocopherylquinone (**5**), α-tocopherylquinone (**6**), and a mixture of four diastereomers of 8a-alkyldioxy-α-tocopherones (**3b**), which were the addition products of the α-tocopheroxyl radical with AMVN-derived peroxyl radicals,[8,9] were detected as oxidation products of α-tocopherol. Identification of these products was based on their chromatographic coelution with authentic standards. Since the concentration of α-tocopherol added to the liposomes was relatively low (0.1 mol%, based on DLPC), only trace amounts of the spirodiene dimer of α-tocopherol, which was not analyzed further, appeared in the reaction mixture (data not shown).[8,14]

Figure 2 shows the results of the formation of PC-OOH, α-tocopherol decay, and production of the oxidation products of α-tocopherol (**3**–**6**) during the AMVN-initiated peroxidation of DLPC liposomes. α-Tocopherol suppress-

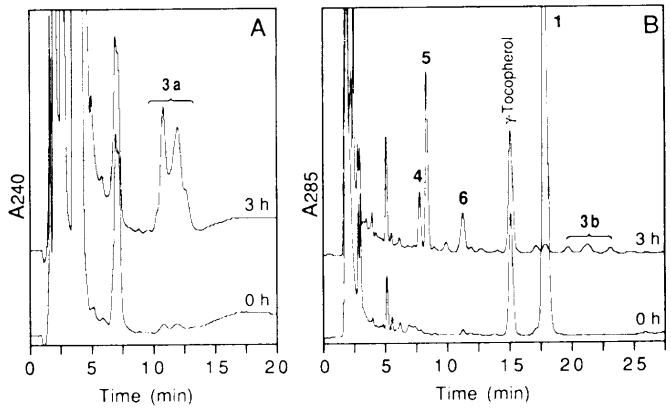

Fig. 1. Reversed-phase HPLC of the Reaction Products of α-Tocopherol in DLPC Liposomes Initiated by AMVN.

Multilamellar liposomes of DLPC (10 mM) containing 0.1 mol% α-tocopherol and 20 mol% AMVN were incubated at 37 °C for 0 and 3 h. (A) Product extracts were analyzed by HPLC without further treatment. HPLC was conducted with an Inertial C8 column (4.6 × 150 mm) developed with methanol–water (99 : 1, v/v). (B) Product extracts were treated by solid-phase extraction before the HPLC analysis as described in the Materials and Methods section. HPLC was conducted with a Puresil C18 column (4.6 × 150 mm) developed with methanol–acetonitrile–water (87 : 10 : 3, v/v).

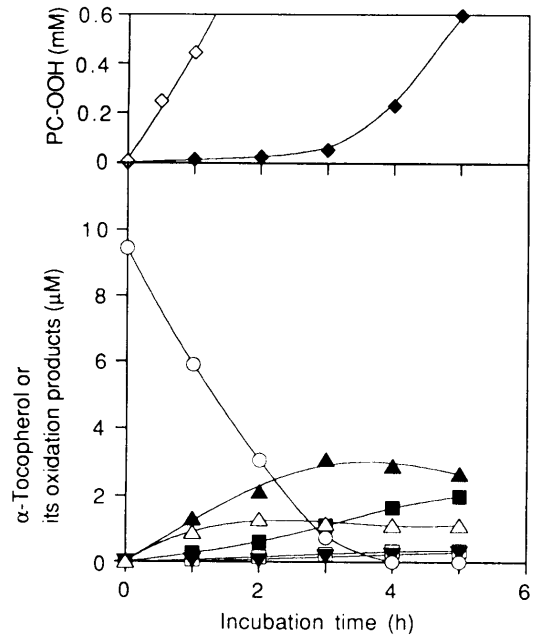

Fig. 2. Reaction of α-Tocopherol during the AMVN-initiated Peroxidation of DLPC.

Multilamellar liposomes of DLPC (10 mM) containing 0.1 mol% α-tocopherol were oxidized by AMVN (20 mol%) at 37 °C. PC-OOH without (◇) or with (◆) α-tocopherol: ○, α-tocopherol; ▲, compound **3a**; △, compound **3b**; □, compound **4**; ■, compound **5**; ▼, compound **6**.

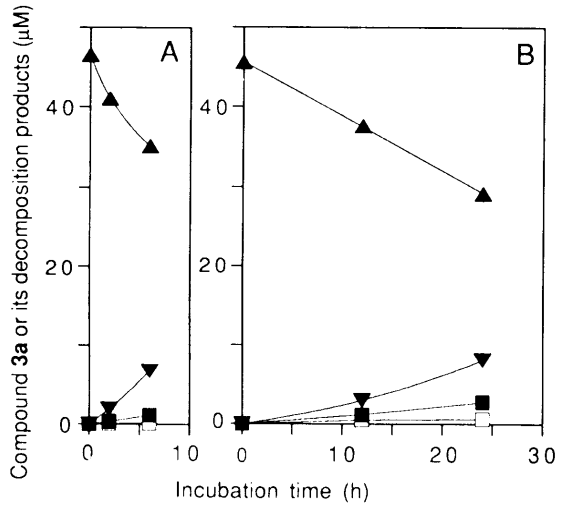

Fig. 3. Decomposition of Compound **3a** in DLPC Liposomes.

Multilamellar liposomes of DLPC (10 mM) containing 0.5 mol% compound **3a** were incubated with (A) or without (B) 20 mol% AMVN at 37 °C. ▲, Compound **3a**; □, compound **4**; ■, compound **5**; ▼, compound **6**.

ed the formation of PC-OOH until the former had disappeared. The main products first formed were DLPC- and AMVN-derived 8a-alkyldioxy-α-tocopherones (**3a** and **3b**), after which epoxy-α-tocopherylquinones (**4** and **5**) and α-tocopherylquinone (**6**) were accumulated in the liposomes.

To evaluate the stability of DLPC-derived 8a-alkyldioxy-α-tocopherones (**3a**), compound **3a** (0.5 mol%, based on DLPC) was incorporated into DLPC liposomes, which were incubated at 37 °C with or without AMVN. Figure 3 shows the fate of **3a** and the formation of its decomposed products in liposomes. Peroxyl radicals derived from AMVN accelerated the decomposition of **3a**. After a 6-h incubation,

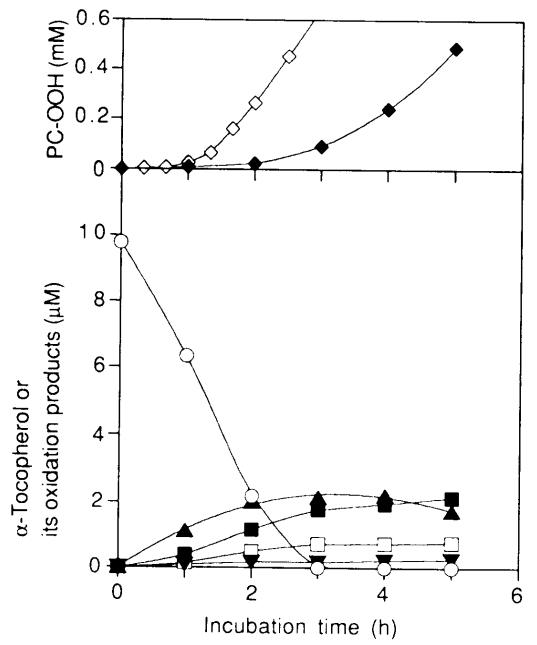

Fig. 4. Reaction of α-Tocopherol during the AAPH-initiated Peroxidation of DLPC.

Unilamellar liposomes of DLPC (10 mM) containing 0.1 mol% α-tocopherol were oxidized by the addition of 2 mM AAPH at 37 °C. PC-OOH without (◇) or with (◆) α-tocopherol: ○, α-tocopherol; ▲, compound **3a**; □, compound **4**; ■, compound **5**; ▼, compound **6**.

approximately 30% of **3a** had been decomposed in the liposomes containing AMVN. On the other hand, the decomposition of **3a** was retarded in the liposomes without AMVN. Thus, **3a** was relatively resistant to hydrolysis in liposomes. The decomposition of **3a** produced primarily α-tocopherylquinone (**6**) and small amounts of epoxy-α-tocopherylquinones (**4** and **5**). Compound **6** arose from simple hydrolysis of **3a**, but the formation of **4** and **5** may have involved further oxidation and hydrolysis of **3a**.[11]

Reaction of α-tocopherol during the peroxidation of DLPC in unilamellar liposomes

Large unilamellar vesicles were prepared by the extrusion method in order to achieve a more realistic model of biological membranes.[23,24] The unilamellar liposomes of DLPC containing 0.1 mol% α-tocopherol were reacted with peroxyl radicals that had been generated in the aqueous phase by the thermal decomposition of AAPH.[3] Figure 4 shows the results of the formation of PC-OOH, α-tocopherol decay, and production of the α-tocopherol oxidation products. α-Tocopherol suppressed the formation of PC-OOH until all the α-tocopherol had been depleted. The products first formed were DLPC-derived 8a-alkyldioxy-α-tocopherones (**3a**), and then epoxy-α-tocopherylquiones (**4** and **5**) and α-tocopherylquinone (**6**) were accumulated in the liposomes. The reaction products between α-tocopherol and the AAPH-derived peroxyl radicals could also be expected, although we could not detect such compounds under the present HPLC conditions.

The transition metals, especially iron and copper, have been strongly implicated as important in the generation of free radicals.[26,27] The transition metal ions may contribute to chain initiation by a variety of mechanisms such as the decomposition of hydroperoxides to give alkoxyl and

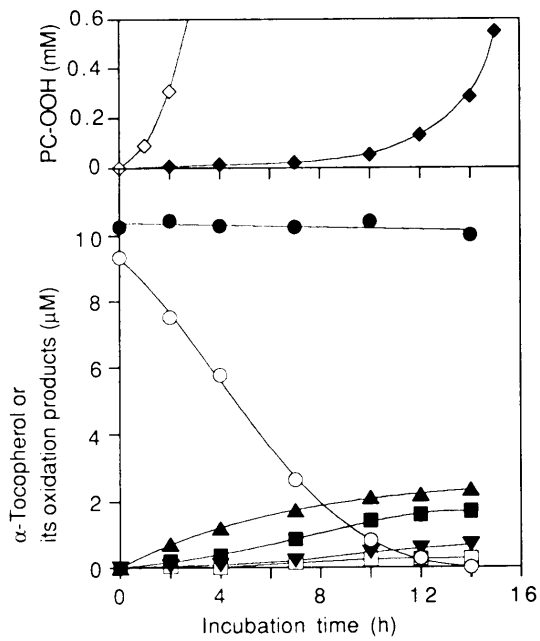

Fig. 5. Reaction of α-Tocopherol during the Peroxidation of DLPC Initiated by $CuSO_4$ and Fructose.

Unilamellar Liposomes of DLPC (10 mM) containing 0.1 mol% α-tocopherol were oxidized by the addition of 10 μM $CuSO_4$ and 10 mM fructose at 37 C. PC-OOH without (◇) or with (◆) α-tocopherol; ○, α-tocopherol; ▲, compound **3a**; □, compound **4**; ■, compound **5**; ▼, compound **6**. DMPC liposomes containing 0.1 mol% α-tocopherol were incubated by the addition of 10 μM $CuSO_4$ and 10 mM fructose at 37 C, and the amounts of α-tocopherol were determined (●).

Table Relative Yields of α-Tocopherol and Its Oxidation Products during the Peroxidation of DLPC in Liposomes[a]

Incubation time (h)	Yield (%)[b]					
	α-Tocopherol	**3a**	**3b**	**4**	**5**	**6**
AMVN-initiated peroxidation						
2	32.4	22.6	13.8	1.6	6.5	0.5
4	nd[c]	30.8	12.1	3.5	17.6	2.4
AAPH-initiated peroxidation						
2	22.4	20.5		5.0	11.8	1.7
4	nd	22.6		7.6	20.0	2.2
Copper-initiated peroxidation						
7	28.4	18.7		1.6	9.3	2.3
14	nd	25.1		2.9	18.2	7.3

[a] These data are from the experiments described in Figs. 2, 4, and 5.
[b] mol% to each theoretical yield is based on the starting material.
[c] nd, not detectable.

peroxyl radicals. Copper is known to be effective in stimulating the decomposition of hydroperoxides.[28] Cu(II) is reduced by reducing sugars, such as fructose, gradually giving Cu(I).[29] Cu(I) is more reactive toward hydroperoxides than Cu(II), and hence the reducing sugar which reduces Cu(II) to Cu(I) may well act as a pro-oxidant.[19,29] Figure 5 shows the results of the copper-initiated peroxidation of DLPC liposomes. α-Tocopherol in the DLPC liposomes suppressed the formation of PC-OOH until the α-tocopherol had disappeared. On the other hand, α-tocopherol in the DMPC liposomes was not oxidized by the addition of $CuSO_4$ and fructose. Thus, α-tocopherol was oxidized by DLPC-peroxyl radicals produced from the copper-dependent peroxidation of DLPC. The oxidation products of α-tocopherol were the same as those observed in the AAPH-initiated oxidation: the main products first formed were 8a-alkyldioxy-α-tocopherones (**3a**), and then epoxy-α-tocopherylquinones (**4** and **5**) and α-tocopherylquinone (**6**) accumulated in the liposomes.

Table compares the relative product yields of α-tocopherol during the peroxidation of DLPC in the liposomes. In all the oxidation system tested, 8a-alkyldioxy-α-tocopherones (**3**) were the main products first formed, and epoxy-α-tocopherylquinones (**4** and **5**) and α-tocopherylquinone (**6**) accumulated with the depletion of α-tocopherol. The oxidation products of α-tocopherol detected in the AMVN-initiated peroxidation accounted for approximately 65% of the consumed α-tocopherol, whereas the oxidation products in the AAPH- and copper-initiated peroxidation processes accounted for 45–50% of the consumed α-tocopherol.

Discussion

α-Tocopherol is known to act as an antioxidant by donating a hydrogen atom to chain-propagating peroxyl radicals. The effectiveness of α-tocopherol depends not only on its ability to trap peroxyl radicals, but also on the ability of the resulting α-tocopheroxyl radical to trap additional peroxyl radicals.[1-3] Therefore, products of the latter trapping reaction may be useful markers for the antioxidative activity of α-tocopherol in a biological system.[30] Studies in a homogeneous solution,[4-9,13] bulk phase,[10,14] and lipid bilayer[11,12,15] indicate that the reaction between the α-tocopheroxyl radical and peroxyl radicals yields several products. Peroxyl radicals add to the 8a-position of α-tocopheroxyl radical in a radical-radical termination reaction to form 8a-alkyldioxy-α-tocopherones.[4,7-11,15] We have already isolated 8a-(phospholipid-dioxy)-α-tocopherones as products of the α-tocopheroxyl radical with DLPC-peroxyl radicals during AMVN-initiated peroxidation in liposomes.[15] However, the 8a-alkyldioxy-α-tocopherones were not the only oxidation products during the peroxidation. In the present study, we detected AMVN-derived 8a-alkyldioxy-α-tocopherones (**3b**), epoxy-α-tocopherylquinones (**4** and **5**), and α-tocopherylquinone (**6**) in addition to 8a-(phospholipid-dioxy)-α-tocopherones (**3a**) (Fig. 1). The similar distribution of these oxidation products among the three oxidation systems indicates that α-tocopherol was oxidized by the same mechanism in the liposomal systems (Table).

The peroxyl radical-trapping reaction of the α-tocopheroxyl radical forms 8a-alkyldioxy-α-tocopherones (**3**), which are then hydrolyzed to α-tocopherylquinone (**6**). The formation of these products, therefore, is consistent with the behavior of α-tocopherol as a chain-breaking antioxidant in liposomes. The decomposition of 8a-alkyldioxy-α-tocopherones (**3**) also yield small amounts of epoxy-α-tocopherylquinones (**4** and **5**) in addition to the major product, α-tocopherylquinone (**6**) (Fig. 3).[11,12] On the contrary, significant amounts of epoxy-α-tocopherylquinones (**4** and **5**) were observed during the peroxidation of DLPC liposomes (Table). This suggests that the formation of expoxy-α-tocopherylquinones occurred along another pathway rather than from the decomposition of 8a-alkyldioxy-α-tocopherones. The formation of epoxy-α-tocopherylquinones and their precursors, epoxyhydroperoxy-α-tocopherones, is thought to involve peroxyl-radical-

dependent epoxidation of the α-tocopheroxyl radical, oxygen addition to the 8a-position, and subsequent hydrogen addition.[6,11] Although the mechanism by which these epoxides were formed remains unknown, the oxidation of α-tocopherol to the epoxides resulted in the net consumption of two peroxyl radicals.[31]

AMVN decomposes at 37°C to form alkyl radicals which react with molecular oxygen to form alkylperoxyl radicals in the hydrophobic inner part of DLPC liposomes.[3] Since DLPC exists in a larger amount than α-tocopherol, most of the AMVN-derived peroxyl radicals would attack DLPC, and the resulting DLPC-peroxyl radicals would react with α-tocopherol.[15] However, both the peroxyl radicals produced from AMVN and DLPC reacted with α-tocopherol to form AMVN- and DLPC-derived 8a-alkyldioxy-α-tocopherones, respectively (Figs. 1 and 2). It is now accepted that α-tocopherol is retained in membranes in such a way that the hydroxyl group is placed at or near the surface and the phytyl side chain is embedded into the membranes,[32] so that the long phytyl side chain must suppress the mobility of α-tocopherol.[33,34] This indicates that α-tocopherol and DLPC, which have long side chains, cannot move as readily as AMVN-derived peroxyl radicals, which do not have a long side chain. Because of the high concentration of DLPC in the liposomes, the first-formed peroxyl radicals from AMVN reacted primarily with DLPC to form the corresponding DLPC-peroxyl radicals. If the mobility of the DLPC-peroxyl radicals within the liposomes is restricted by their fatty acyl chains, the reaction between the DLPC-peroxyl radicals and α-tocopherol must be suppressed. Consequently, the AMVN-derived peroxyl radicals, as well as DLPC-peroxyl radicals, could react with α-tocopherol to form the corresponding 8a-alkyldioxy-α-tocopherones. The AAPH-derived peroxyl radicals produced in the aqueous phase may also have been scavenged by α-tocopherol before the reaction with DLPC to form AAPH-derived 8a-alkyldioxy-α-tocopherones. Although we could not detect AAPH-derived tocopherones, the low yield of the oxidation products from the AAPH-initiated oxidation compared with that from the AMVN-initiated oxidation might indicate the formation of such products.

Ham and Liebler[30] have identified the oxidation products of α-tocopherol in rat liver mitochondria during AAPH-initiated peroxidation. The principal products formed were epoxy-α-tocopherylquinones and α-tocopherylquinone, these accounting for approximately 80% of the consumed α-tocopherol. Their results indicate that antioxidative reactions of α-tocopherol in mitochondria are similar to those identified in liposomal systems.[11,12] However, no evidence for the formation of 8a-alkyldioxy-α-tocopherones was found in rat liver mitochondria. Further studies on the identification of oxidation products of α-tocopherol on membranes are needed to elucidate the antioxidative activity of α-tocopherol in biological systems.

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