Structural and Dynamic Membrane Properties of α-Tocopherol and α-Tocotrienol: Implication to the Molecular Mechanism of Their Antioxidant Potency†

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ABSTRACT: *d*-α-Tocopherol and *d*-α-tocotrienol are two vitamin E constituents having the same aromatic chromanol "head" but different hydrocarbon "tails". α-Tocotrienol has been shown to be more potent in protecting against free radical-induced oxidative stress than α-tocopherol. Simple models of phospholipid membrane systems were used to investigate the mechanism of the antioxidant potency of α-tocotrienol in terms of its effects on membrane order and reorientational dynamics. Chemiluminescence and fluorescence measurements demonstrated that α-tocotrienol exhibits significantly greater peroxyl radical scavenging potency than α-tocopherol in phosphatidylcholine liposomes, whereas both antioxidants have identical activity in hexane. This suggests that the antioxidant potency of α-tocotrienol requires the membrane environment. When α-tocopherol and α-tocotrienol were examined for their effects on phospholipid molecular order using conventional ESR spin labeling with 5- and 16-position-labeled doxylstearic acid, although both vitamin E constituents disordered the gel phase and stabilized the liquid-crystalline phase, no differences were observed between the effects of the two compounds. A slightly greater increase (19% vs 15%) in ordering of the liquid-crystalline state due to α-tocopherol, however, was discerned in noninvasive ^{2}H NMR experiments. The difference is most noticeable near C10–C13 positions of the phospholipid chain, possibly suggesting α-tocotrienol is located closer to the membrane surface. Saturation-transfer ESR, furthermore, revealed that on the time scale $\tau_c = 10^{-7}$–10^{-3} s the rates of rotation about the long molecular axis and of the wobbling motion of the axis are modified to differing extents by the two forms of the vitamin E. Calculation of the ratio of correlation times $\tau_c(L''L)$ and $\tau_c(C'/C)$ associated with the two motions suggests that α-tocotrienol imposes more motional anisotropy on the membrane. Thus, the different effects of α-tocotrienol on the molecular properties of the membrane may explain its greater antioxidant potency.

Vitamin E is a lipid component of biological membranes and is considered to be a major-chain breaking antioxidant (Burton et al., 1980). *d*-α-Tocopherol and *d*-α-tocotrienol are two forms of vitamin E having the same aromatic chromanol "head" but different hydrocarbon "tails": α-tocopherol has a saturated hydrocarbon "tail" and α-tocotrienol an unsaturated "tail". Recent studies suggest that α-tocotrienol exerts higher efficiency in protecting against some free radical-related diseases than does α-tocopherol (Tatsuta, 1971; Kato et al., 1985; Sundram et al., 1989; Komiyama et al., 1989). A more direct evidence of the better efficacy of α-tocotrienol as an antioxidant has been reported recently (Serbinova et al., 1991). α-Tocotrienol exhibits greater antioxidant activity against lipid peroxidation in rat liver microsomal membranes and greater protection from oxidative damage of cytochrome P-450. Further studies suggest that this higher antioxidant potency of α-tocotrienol may be due to the combined effects of its more uniform distribution in the membrane and its stronger disordering of membrane lipids, which result in its higher recycling efficiency (Serbinova et al., 1991). Yamaoka and Carrillo (1990) also observed a difference in the changes in physicochemical properties of the membrane induced by α-tocotrienol and α-tocopherol.

In addition to its role as an antioxidant, α-tocopherol has been shown to affect membrane architecture and its dynamic properties (Srivastava et al., 1983; Urano et al., 1988; Wassall et al., 1986, 1989; Ekiel et al., 1988). These reports indicate a possible role of vitamin E–membrane interactions in the mechanisms of antioxidant action and membrane protection. Similarly, such a notion may apply to a possible mechanism of the difference between the antioxidant potencies of α-tocotrienol and α-tocopherol. Unsaturation in the tail of α-tocopherol should confer a different three-dimensional molecular structure of α-tocotrienol, and this may have a pronounced effect on the orientation and organization of membrane phospholipids. Changes in membrane properties by unsaturation of phospholipid have been reported (Stubbs et al., 1981; Deinum et al., 1988). As an extension of the studies by Serbinova et al. (1991) and Yamaoka and Carrillo (1990), we report the structural and dynamic membrane properties of α-tocotrienol as compared to α-tocopherol, which may be responsible for differences in their antioxidant potency.

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A simple model of phospholipid membrane systems was studied in terms of the effects of α-tocopherol and α-tocotrienol on membrane molecular ordering and reorientational dynamics using NMR[1] and ESR spectroscopy.

MATERIALS AND METHODS

HPLC Measurements. Purity of α-tocopherol (Sigma) and α-tocotrienol (Palm Oil Research Institute of Malaysia) was determined by HPLC with in-line electrochemical detection (Beckman 114M solvent delivery module, Beckman 340 organizer, Beckman ultrasphere ODS 5 μm 4.6 mm × 25 cm, Shimadzu CR501 Chromatopac, Bioanalytical System LC4b amperometric detector) as previously described (Lang et al., 1986). Both the α-tocopherol and the α-tocotrienol preparations used in all experiments eluted with a single peak at 4.6 and 4.0 min, respectively.

Distributions of α-tocopherol and α-tocotrienol between liposomes and aqueous solution were determined by HPLC. Ten milligrams of dipalmitoylphosphatidylcholine (DPPC; Sigma) dissolved in chloroform and α-tocopherol or α-tocotrienol dissolved in ethanol (20 mol % DPPC) was mixed in a round-bottomed glass tube. A dried lipid film was obtained by evaporation with a nitrogen flux, and the film was hydrated with borate buffer (pH 9.4). The suspension was centrifuged at 14000*g*, and vitamin E in the liposomal phase was extracted in 1:2:2 H_2O/ethanol/hexane. The hexane phase was dried, reconstituted in ethanol, and then subjected to HPLC measurements.

cis-Parinaric Acid Fluorescence-Based Assay for Peroxyl Radical Scavenging Activity. The assay was performed at 40 °C using a hydrophobic azo-initiator of radicals, 2,2′-azobis-(2,4-dimethylvaleronitrile) $[CH(CH_3)_2CH_2C(CH_3)$-$CNN{=}NC(CH_3)CNCH_2CH(CH_3)_2]$ (AMVN; Polysciences Inc.) and a polyunsaturated fatty acid, *cis*-parinaric acid (Molecular Probes), as described by Tsuchiya et al. (1992). AMVN produces peroxyl radicals (ROO·) at a constant rate by thermal decomposition as follows:

$$RN{=}NR \rightarrow [R^{\bullet}\ N_2\ {}^{\bullet}R] \rightarrow 2eR^{\bullet} + (1-e)RR + N_2$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

Oxidation of *cis*-parinaric acid by peroxyl radicals was monitored by a decay of its characteristic fluorescence using a Perkin-Elmer MPF-44A fluorescence spectrophotometer. Peroxyl radical scavenging activities of the two vitamin E constituents were measured in hexane solution and in DPPC liposomes. DPPC liposomes were prepared by sonication of 2.5 mM DPPC dispersion in 20 mM Tris-HCl (pH 7.4) under nitrogen gas at 4 °C. The liposomes were further sonicated to incorporate α-tocopherol or α-tocotrienol (Kagan et al., 1991).

Chemiluminescence Assay for Peroxyl Radical Scavenging Activity in Liposomes. DPPC or dioleoylphosphatidylcholine (DOPC; Sigma) liposomes with incorporated α-tocopherol or α-tocotrienol were made by sonication as described above. The incubation medium (2 mL) contained DPPC or DOPC liposomes (2.5 mM), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma) (150 μM), and α-tocopherol or α-tocotrienol in Tris-HCl buffer (pH 7.4). The reaction was started at 40 °C by the addition of AMVN (final concentration 2.5 mM). Chemiluminescence signals were measured using a Pharmacia-LKB Wallac 1250 luminometer equipped with a BioOrbit dispenser.

Measurement of Oxygen Consumption Induced by Lipid Peroxidation. Oxygen consumption in the course of lipid peroxidation was determined by an oxygen electrode (Niki, 1990) at 40 °C. Incubation mixture contained dilinoleoylphosphatidylcholine (5 mM; DLPC), AMVN (5 mM), NaCl (0.1 M), and various concentrations of α-tocopherol or α-tocotrienol in 20 mM Tris-HCl (pH 7.4).

^2H NMR Spectroscopy. Samples were aqueous multilamellar dispersions of [$^2H_{31}$]16:0–16:0 PC (1-[$^2H_{31}$]palmitoyl-2-palmitoylphosphatidylcholine) (Avanti Polar Lipids, Inc., Alabaster, AL) with and without α-tocopherol or α-tocotrienol in 50% by wt 20 mM Tris buffer (pH 7.5). They contained approximately 100 mg of lipid. The method of preparation was essentially as described previously (Wassall et al., 1986), except that three lyophilizations with excess deuterium-depleted water (Sigma) were performed to reduce the ^2H NMR signal from residual ^2HHO.

^2H NMR spectra were recorded on a home-built broad-line spectrometer operating at 27.6 MHz (Wassall et al., 1989). Implementation of the quadrupolar echo sequence ($90°_x$–τ_2–$90°_y$–acquire–$T)_n$ eliminated spectral distortion due to receiver recovery time (Davis, 1983). The 90° pulse width was of the order of 2.5 μs, the time decay τ_2 between pulses was 50 μs unless otherwise stated, and the delay T between sequence repetition was in the range 1.0–1.5 s. Spectral parameters were as follows: sweep width = ±250 and ±100 kHz in gel and liquid-crystalline phases, respectively; line broadening = 125 and 50 Hz in the respective phases; data set = 2048; and the number of scans was usually 1024. The accumulated NMR signals were transferred to Zenith 386 or Renaissance 486 computers for Fourier transformation and subsequent data analysis. Spectral moments M_n were calculated and related to average order parameters $\bar{S}_{CD}$ with standard equations (Davis, 1983). Numerical deconvolution ("depaking") of the powder pattern spectra in the liquid-crystalline state produced "aligned" spectra possessing enhanced resolution (Sternin et al., 1983), from which smoothed order parameter S_{CD} profiles were constructed on the assumption that S_{CD} depends monotonically on acyl chain position (Lafleur et al., 1989).

ESR Spin Labeling. Stearic acid-labeled DPPC liposomes were prepared as described by Severcan and Cannistraro (1990). Ten milligrams of DPPC dissolved in chloroform, the fatty acid nitroxide spin probes 5-doxylstearic acid (5-DSA; Aldrich) or 16-doxylstearic acid (16-DSA; Aldrich) dissolved in ethanol (1 mol % DPPC), and α-tocopherol or α-tocotrienol dissolved in ethanol (20 mol % DPPC) were mixed in a round-bottomed glass tube. A dried lipid film was obtained by evaporation with a nitrogen flux and pumping overnight under vacuum. The film was hydrated with 200 μL of borate buffer at pH 9.4.

Samples were placed in 50 μL of Accupette Pipets (Baxter) sealed with Sure-SEAL tube sealant (Baxter) within standard 4-mm diameter quartz tubes.

ESR Spectroscopy. ESR spectra were recorded at 9.2 GHz on a Bruker ER200D-SRC electron spin resonance spectrometer. Submicrosecond rotational motion of spin labels was detected by conventional ESR (first harmonic absorption

[1] Abbreviations: AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); DLPC, dilinoleoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; 5-DSA, 5-doxylstearic acid; 16-DSA, 16-doxylstearic acid; ESR, electron spin resonance; ^2H NMR, deuterium nuclear magnetic resonance; [$^2H_{31}$]16:0–16:0 PC, 1-[$^2H_{31}$]palmitoyl-2-palmitoyl-phosphatidylcholine; M_n, spectral moment; ROO·, peroxyl radical; S, order parameter (ESR spin labeling); S_{CD}, order parameter (^2H NMR); $\bar{S}_{CD}$ average order parameter; τ_2, time decay; τ_c, correlation time.

in phase, designated V_1) using 100-kHz field modulation frequency, 2-G modulation amplitude, and 9.9-mW microwave power. Submillisecond rotational motion was detected by saturation-transfer ESR (second harmonic absorption out of phase, designated V_2') using 50-kHz field modulation, 5-G modulation amplitude, and 63-mW microwave power (Squier & Thomas, 1986).

ESR Spectral Analysis. The order parameter S for 5-DSA-labeled samples was calculated from the conventional ESR spectra according to Hubbell and McConnell (1971):

$$S = (T_{\parallel} - T_{\perp})/[T_{zz} - (T_{xx} + T_{yy})/2]$$

where $2T_{\parallel}$ and $2T_{\perp}$ are the outer and the inner hyperfine splitting and T_{xx}, T_{yy}, and T_{zz} are the principle hyperfine coupling constants of the spin probe (see Figure 7). For the 16-DSA-labeled sample, inner and outer extrema were not resolved because the membrane is highly disordered at the 16-position. Correlation times τ_c for the approximately isotropic motion were calculated from line width W_0 as described by Eletr and Inesi (1972):

$$\tau_c = (6.5 \times 10^{-10}) W_0[(h_0/h_{-1})^{1/2} - 1]$$

where h refers to first derivative line heights (see Figure 8). The equations for S and τ_c apply in the liquid-crystalline phase. Spectral interpretation in the gel phase is qualitative.

Central, C'/C, and low, L''/L, field peak height ratios were measured from saturation transfer ESR spectra analysis (Thomas et al., 1976) (see Figure 10). The associated correlation times τ_c(C'/C) and τ_c(L''/L) were obtained from the calibration curves reported in Horvath and Marsh (1988). Strictly speaking, the calibrations refer to isotropic motion, which is not the case in the inherently anisotropic environment of the membrane. The values derived for τ_c(C'/C), which describes motion around the long molecular axis, and τ_c(L''/L), which describes motion of the long axis itself, consequently differ. The difference is an indication of the anisotropy in the motion (Marsh, 1980; Robinson & Dalton, 1980).

RESULTS

Distribution of α-Tocopherol and α-Tocotrienol in Liposomal and Aqueous Phases. HPLC measurements show that contents of α-tocopherol and α-tocotrienol extracted from the liposomal phase of DPPC liposome/buffered solution system correspond to 4.22 ± 0.26 and 3.77 ± 0.29 ($n = 5$) μM, respectively. These values are not significantly different from each other at $P < 0.05$.

Peroxyl Radical Scavenging Activities of α-Tocopherol and α-Tocotrienol. The peroxyl radical scavenging activities of α-tocopherol and α-tocotrienol were measured by monitoring AMVN-induced loss of fluorescence of *cis*-parinaric acid in DPPC liposomes and in hexane solution. In liposomes, *cis*-parinaric acid has its maximum fluorescence in the excitation spectrum at 328 nm and in the emission spectrum at 415 nm (Kuypers et al., 1987). The oxidative loss of *cis*-parinaric acid fluorescence is partially prevented by the addition of vitamin E (Figure 1). α-Tocotrienol is more effective than α-tocopherol. On the other hand, in hexane solution, the two vitamin E constituents are equally effective (Figure 2).

Using a chemiluminescence technique, consistent results were obtained after addition of AMVN to DPPC or DOPC liposomes, which produced a characteristic luminol-amplified chemiluminescence. This response was not observed in the absence of liposomes (data not shown), indicating that the chemiluminescence signals represent the reaction of AMVN-derived peroxyl radicals with luminol in liposomes. Both α-tocopherol and α-tocotrienol quench the AMVN-induced luminol-enhanced chemiluminescence in DPPC liposomes (Figure 3) with half quenching concentrations of 50 and 15 nM for α-tocopherol and α-tocotrienol, respectively. Similar results were obtained in DOPC liposomes (data not shown). These indicate that α-tocotrienol is a more efficient scavenger of peroxyl radicals than α-tocopherol in membranes.

The induction periods of oxygen consumption, by the same concentration of α-tocopherol and α-tocotrienol in the course of AMVN-induced oxidation of DLPC liposomes, were not found to be significantly different (data not shown), suggesting

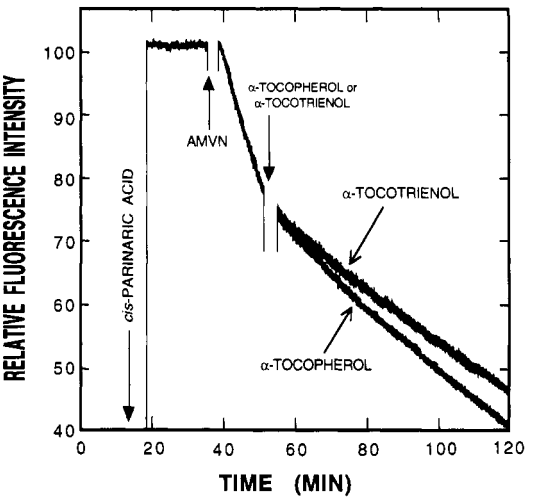

FIGURE 1: *cis*-Parinaric acid assay for comparison of the antioxidant activity of α-tocopherol and α-tocotrienol in DPPC liposomes. Generation of peroxyl radicals by AMVN was detected by monitoring decay of fluorescence of *cis*-parinaric acid (λ_{excit} 328 nm, λ_{emiss} 415 nm). The reaction mixture (2 mL) contained AMVN (500 μM) and *cis*-parinaric acid (18 μM) in 20 mM Tris-HCl (pH 7.4). α-Tocopherol or α-tocotrienol (1.5 μM) dissolved in ethanol was incorporated into the membrane by further sonicating the DPPC liposomes, which were initially prepared by sonication with AMVN under nitrogen. Temperature = 40 °C.

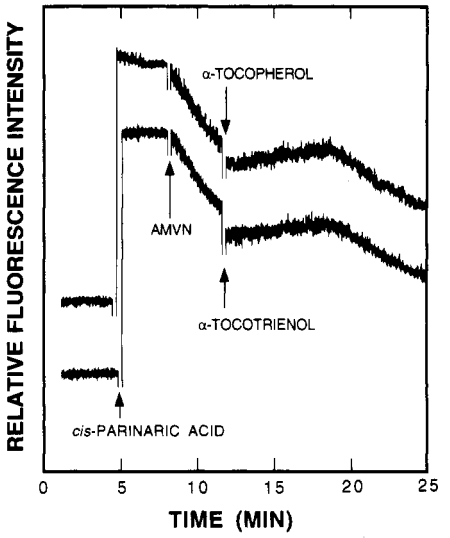

FIGURE 2: *cis*-Parinaric acid assay for comparison of the antioxidant activity of α-tocopherol and α-tocotrienol in hexane. Generation of peroxyl radicals by AMVN was detected by monitoring decay of fluorescence of *cis*-parinaric acid (λ_{excit} 304 nm, λ_{emiss} 421 nm). The reaction mixture (3 mL) contained AMVN (100 mM) and *cis*-parinaric acid (100 μM). α-Tocopherol or α-tocotrienol (60 μM) dissolved in chloroform was added to the incubation medium during the course of AMVN-induced fluorescence loss of *cis*-parinaric acid. Temperature = 40 °C.

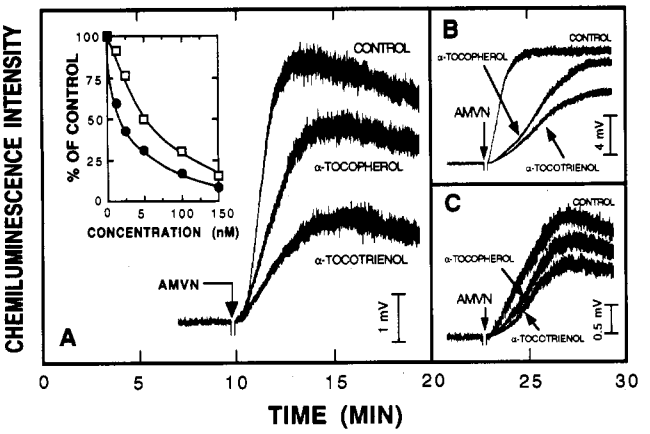

FIGURE 3: Chemiluminescence assay for comparison of the antioxidant activity of α-tocopherol and α-tocotrienol in DPPC liposomes. Generation of peroxyl radicals by AMVN (2.5 mM) was detected by monitoring the chemiluminescence of luminol (150 μM) at various temperatures. (A) 40 °C, [vitamin E] = 25 nM; (B) 50 °C, [vitamin E] = 200 nM; (C) 30 °C, [vitamin E] = 5 nM.

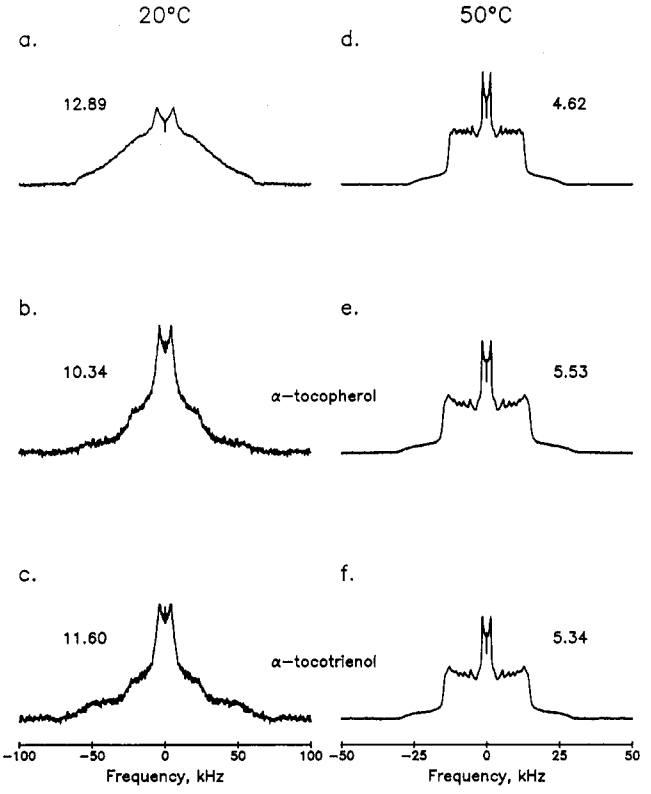

FIGURE 4: ^2H NMR spectra for [$^2H_{31}$]16:0–16:0 PC (a and d), [$^2H_{31}$]16:0–16:0 PC/α-tocopherol, 4:1 molar ratio (b and e), and [$^2H_{31}$]16:0–16:0 PC/α-tocotrienol, 4:1 molar ratio (c and f) at 20 and 50 °C. The samples were multilamellar liposomes of approximately 100 mg of lipid in 50% by wt 20 mM Tris (pH 7.5). Spectral parameters are as described in Materials and Methods. First moments M_1 (10^4 s^{-1}) are stated next to the spectra.

that the stoichiometric numbers of ROO· trapped by the two vitamin E constituents are identical (Niki et al., 1986).

^2H NMR Studies. ^2H NMR spectra collected for aqueous multilamellar dispersions of [$^2H_{31}$]16:0–16:0 PC, [$^2H_{31}$]16:0–16:0 PC/α-tocopherol (4:1), and [$^2H_{31}$]16:0–16:0 PC/α-tocotrienol (4:1) are compared in Figure 4. At 20 °C they are representative of the gel state but differ in shape (Figure 4a–c). Relative intensity in the "wings" of the spectrum with edges at ±63 kHz is less in the presence of α-tocopherol or α-tocotrienol, and an intermediate component of approximately 50 kHz in width becomes discernible. Similar behavior

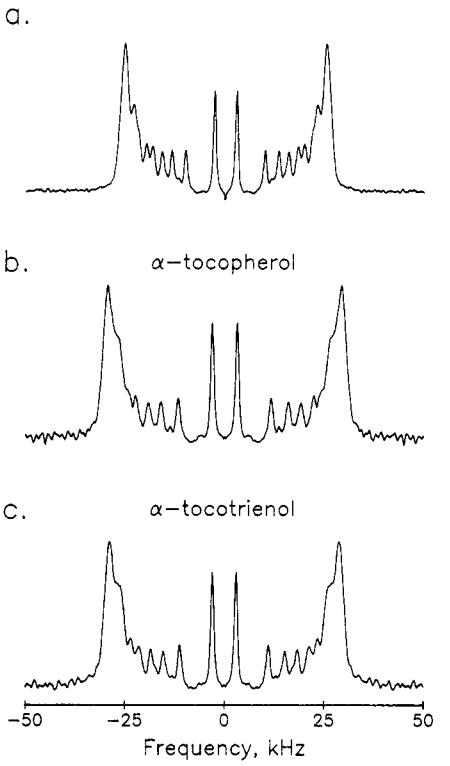

FIGURE 5: Depaked ^2H NMR spectra for [$^2H_{31}$]16:0–16:0 PC (a), [$^2H_{31}$]16:0–16:0 PC/α-tocopherol, 4:1 molar ratio (b), and [$^2H_{31}$]-16:0–16:0 PC/α-tocotrienol, 4:1 molar ratio (c) at 50 °C. The samples were multilamellar liposomes of approximately 100 mg of lipid in 50% by wt 20 mM Tris (pH 7.5). Spectral parameters are as described in Materials and Methods. Typically, 6 iterations of the depaking program were performed.

was seen earlier for α-tocopherol in PC-d_{31} (*sn*-2-[$^2H_{31}$]16:0-substituted egg PC) bilayers and in [$^2H_{62}$]16:0–16:0 PC bilayers containing free fatty acid (Wassall et al., 1986, 1990). It indicates that both forms of the vitamin disrupt molecular packing within gel state membranes, resulting in additional acyl chain motion. First moments M_1 are noted in Figure 4, and the larger reduction measured with α-tocopherol (20% vs 10%) suggests that disruption is somewhat greater than with α-tocotrienol.

Typical liquid-crystalline phase spectra were obtained at 50 °C (Figure 4d–f). The well-defined, sharp edges at approximately ±15 kHz are indicative of the plateau region of almost constant order in the upper portion (C2–9) of the chain, while the peaks are associated with individual segments in the lower portion (C10–16) where order gradually decreases toward the terminal methyl. The introduction of α-tocopherol or α-tocotrienol broadens both the overall spectrum and the peaks. This agrees with the previous ^2H NMR work which showed that vitamin E increases order in liquid crystalline bilayers (Wassall et al., 1986, 1993). The magnitude of the increase is quantified by average order parameters $\overline{S}_{CD}$ calculated from first moments M_1 (Figure 4), which reveal that α-tocopherol causes slightly more ordering (19% vs 15%) than α-tocotrienol. The corresponding depaked spectra are presented in Figure 5 and were employed to construct the smoothed order parameter profiles plotted in Figure 6. They confirm the trend seen in average order $\overline{S}_{CD}$, demonstrating that both forms of vitamin E increase order throughout the phospholipid chains. The profiles elaborate that the distinction between their effect is not uniform along the chain. Instead the greater, albeit subtle, increase due to α-tocopherol is predominantly manifest at positions C10–13.

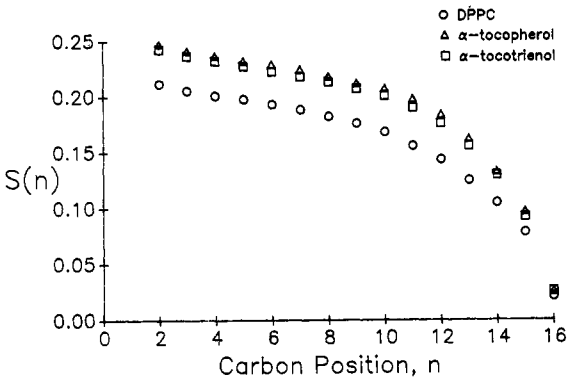

FIGURE 6: Order parameter profiles for [$^2H_{31}$]16:0–16:0 PC, [$^2H_{31}$]-16:0–16:0 PC/α-tocopherol (4:1), and [$^2H_{31}$]16:0–16:0 PC/α-tocotrienol (4:1) membranes at 50 °C.

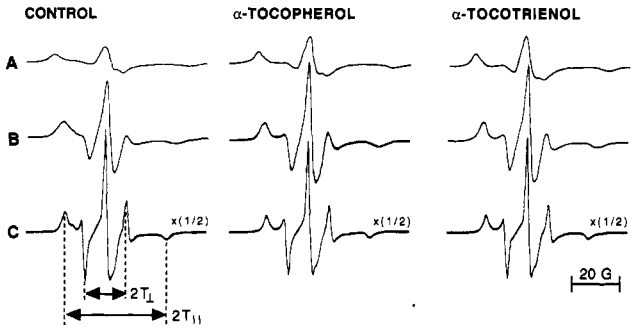

FIGURE 7: Conventional ESR spectra of 5-DSA at (A) 5 °C, (B) 30 °C and (C) 60° C. [DPPC] = 68 mM; [5-doxylstearic acid (5-DSA)] = 0.68 mM; [α-tocopherol] = 13.6 mM; [α-tocotrienol] = 13.6 mM.

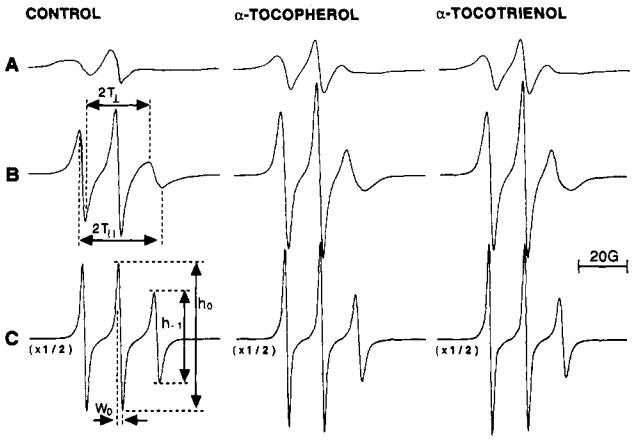

FIGURE 8: Conventional ESR spectra of 16-DSA at (A) 5 °C, (B) 30 °C, and (C) 60 °C. [DPPC] = 68 mM; [16-doxylstearic acid (16-DSA)] = 0.68 mM; [α-tocopherol] = 13.6 mM; [α-tocotrienol] = 13.6 mM.

Conventional ESR Spin Labeling Studies. Conventional ESR spectra of 5-DSA (Figure 7) and 16-DSA (Figure 8) spin-labeled DPPC liposomes at 5, 30, and 60 °C show that, at all temperatures, control spectra differ from those in which the vitamin E constituents were present. Comparable spectral narrowing is observed below the gel to liquid-crystalline phase transition with α-tocopherol and α-tocotrienol. This indicates that they produce a similar increase in lipid mobility in the gel phase. Order parameters S and motion parameter τ_c calculated for the 5- and 16-positions, respectively, above the phase transition reveal that both forms of the vitamin increase order and decrease fluidity to a similar extent in the liquid-crystalline state (Figure 9). These results are consistent with previous ESR reports on α-tocopherol (Severcan & Cannistraro, 1990; Wassall et al., 1991). However, although the influence of α-tocopherol and α-tocotrienol on acyl chain order dynamics in DPPC membranes is clearly seen, there are no apparent differences in their effects as detected by conventional ESR.

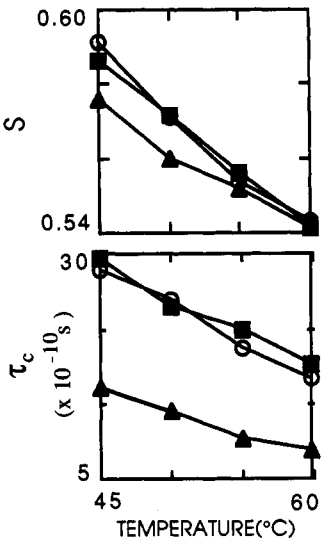

FIGURE 9: Temperature dependence of order parameter and correlation time from conventional ESR spectra of 5- and 16-DSA. [DPPC] = 68 mM; [5-doxylstearic acid (5-DSA)] = 0.68 mM; [16-doxylstearic acid (16-DSA)] = 0.68 mM; [α-tocopherol] = 13.6 mM; [α-tocotrienol] = 13.6 mM. Triangles: control; circles:α-tocopherol; squares: α-tocotrienol

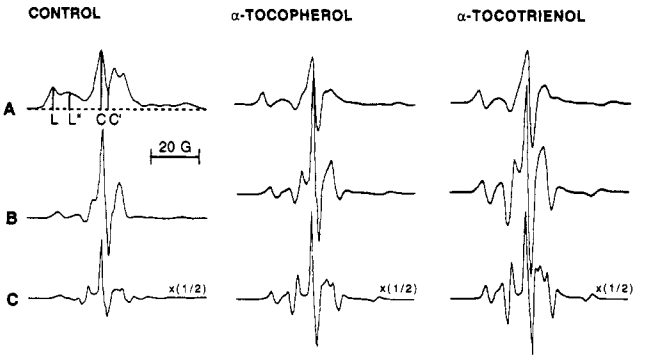

FIGURE 10: Saturation-transfer ESR spectra of 5-DSA at (A) 5 °C, (B) 30 °C, and (C) 60 °C. [DPPC] = 68 mM; [5-doxylstearic acid (5-DSA)] = 0.68 mM; [α-tocopherol] = 13.6 mM; [α-tocotrienol] = 13.6 mM.

Saturation-Transfer ESR Spin Labeling Studies. Slow motions of 5-DSA spin labels in DPPC liposomes were detected using saturation transfer ESR. The temperature dependencies of central-field (C/C) and low-field (L/L) parameters, calculated using the spectra shown in Figure 10, are plotted in Figure 11. Both parameters are lower and higher in the gel and liquid-crystalline phases, respectively, for preparations containing α-tocopherol or α-tocotrienol than in preparations without vitamin E (control). These observations indicate that the addition of either form of the vitamin increases the rate of motion around the long axis of the spin-labeled fatty acid (C′/C) and the rate of reorientation of the axis (L″/L) in the gel phase, whereas in the liquid crystalline phase the rates of the two motions are substantially reduced. This is qualitatively consistent with saturation transfer ESR results on α-tocopherol by Severcan and Cannistraro (1990).

The behavior monitored in Figure 11 for α-tocopherol vs α-tocotrienol, although similar, clearly differs in detail. The values of C′/C are lower, with α-tocotrienol predominantly

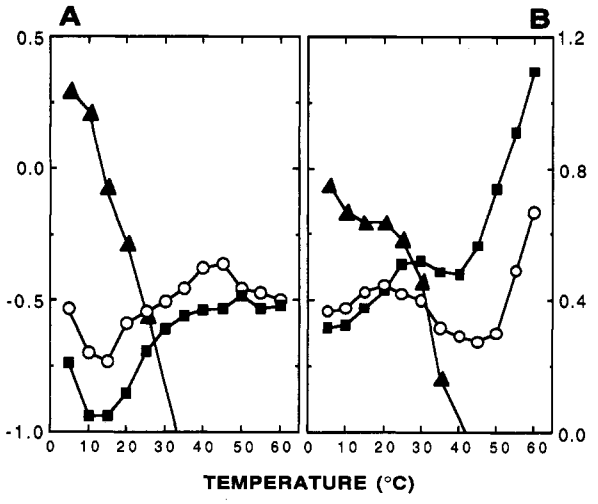

FIGURE 11: Temperature dependence of parameters (A) C′/C and (B) L″/L from saturation-transfer ESR spectra of 5-DSA. [DPPC] = 68 mM; [5-doxylstearic acid (5-DSA)] = 0.68 mM; [α-tocopherol] = 13.6 mM; [α-tocotrienol] = 13.6 mM. Triangles: control; circles: α-tocopherol; squares: α-tocotrienol.

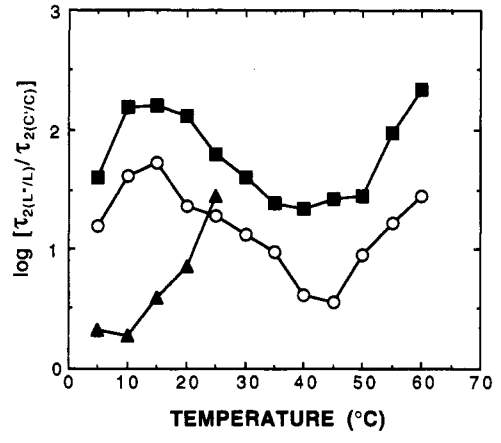

FIGURE 12: Temperature dependence of log of the ratio of correlation times derived from L″L and C′/C. [DPPC] = 68 mM; [5-doxylstearic acid (5-DSA)] = 0.68 mM; [α-tocopherol] = 13.6 mM; [α-tocotrienol] = 13.6 mM. Triangles: control; circles:α-tocopherol; squares: α-tocotrienol.

in the gel state; while the higher values of L″/L occur predominantly in the liquid-crystalline state. The plot of log-$[\tau_c(L''/L)/\tau_c(C'/C)]$ vs temperature in Figure 12 elaborates on the differential effects on the rates of motion detected for the two forms of the vitamin. As concluded in earlier work (Severcan & Cannistraro, 1990), the introduction of α-tocopherol is seen to increase the anisotropy of motion in the gel phase. Our data, moreover, establish that the motional anisotropy is larger with α-tocotrienol. Thus, a distinction between the effects of α-tocopherol and α-tocotrienol on the properties of membranes at the molecular level is identified.

DISCUSSION

Although α-tocotrienol is known to possess a lower biological "vitamin E activity" than α-tocopherol (Leth & Sondergaad, 1977), recent findings suggest that α-tocotrienol is a better "antioxidant" (Serbinova et al., 1991). Our results confirm the report of Serbinova et al. and further demonstrate that α-tocotrienol is more potent in scavenging peroxyl radicals in membranes. A higher antioxidant potency of α-tocotrienol is also commensurate with the recent finding that the hearts from rats supplemented with α-tocotrienol-rich diet were more resistant against oxidative damage induced by ischemia–reperfusion (Serbinova et al., 1992).

To examine the importance of membranes in evaluating the relative antioxidant potency of α-tocotrienol and α-tocopherol, we first compared their peroxyl radical scavenging potency in solution to that observed in the presence of liposomes. α-Tocotrienol exhibited a greater potency than α-tocopherol in eliminating the chemiluminescence and fluorescence characteristics resulting from AMVN-generated ROO· in liposomes. However, the quenching effects were similar in solution. Since the effects on induction period of O_2 consumption were not different for the two vitamin E constituents, α-tocotrienol appears to possess an identical stoichiometric number to trap ROO· (Niki et al., 1986), yet different quenching efficacy as compared to α-tocopherol.

We have previously suggested that α-tocotrienol has a strong disordering effect on membrane lipids which makes interaction of chromanols with lipid radicals more efficient (Serbinova et al., 1991). Suggestions by Serbinova et al. (1991), however, were based on the observations of the temperature-dependent changes in the height of the ^1H NMR spectral peak from methylene protons in DMPC-sonicated vesicles. This spectral peak is a superposition of signals with a range of line widths from methylenes throughout the phospholipid chain. Unambiguous interpretation in terms of membrane organization requires line-shape analysis (Bloom et al., 1978). Thus, a suggestion based solely on signal intensity is questionable, and further investigations are necessary. We have extended their work using NMR and ESR spectroscopy to answer whether structural differences between these compounds lead α-tocotrienol to exert different effects on the structure and dynamics of membranes, which may cause α-tocotrienol to possess higher mobility through the membranes and thereby leading to a greater radical scavenging potency of α-tocotrienol compared to α-tocopherol.

Conventional ESR of 5- and 16-DSA in DPPC membranes clearly demonstrates that incorporation of α-tocopherol or α-tocotrienol modifies acyl chain ordering and reorientational dynamics within the membrane. Lipid mobility is increased in the gel phase, whereas in the liquid-crystalline phase order is increased and the rate of acyl chain reorientation is decreased. No distinction between the effects of the two forms of the vitamin is detected. This finding is consistent with earlier fluorescence polarization measurements on 1,6-diphenyl-1,3,5-hexatriene in DOPC membranes, which detected comparable changes in ordering due to α-tocopherol or α-tocotrienol (Yamaoka & Carrillo, 1990). The polarization values measured by Yamaoka and Carrillo, however, were relatively low. It has been shown that the determination of molecular order under such conditions is highly inaccurate (Pottel et al., 1983).

^2H NMR results, which by their essentially noninvasive nature possess an advantage over ESR and fluorescence observations involving bulky extrinsic probes, are in qualitative agreement. They indicate that the vitamin E constituents disturb molecular packing in the gel phase, while in the liquid crystalline state order is increased. Small differences between the two forms of the vitamin, however, are seen. In particular, average order in the liquid crystalline state at 50 °C ($\bar{S}_{CD}$ = 0.152) is increased by 19% with 20 mol % α-tocopherol ($\bar{S}_{CD}$ = 0.181) as compared to 15% for 20 mol % α-tocotrienol ($\bar{S}_{CD}$ = 0.175). The order parameter profiles constructed in Figure 6 from depaked spectra (Figure 5) confirm that α-tocopherol increases order more and reveal that the greater order is sensed preferentially in the C10–13 portion of the phospholipid chain. This intriguingly may suggest that α-tocotrienol resides closer to the surface of the

membrane and perhaps relates to its superior efficacy as a membrane antioxidant.

It has been noted that molecular ordering of the hydrocarbon chains must be considered separately from reorientational dynamics (Seelig & Seelig, 1977; Korstanje et al., 1989). Our conventional ESR and ^{2}H NMR experiments were largely concerned with measuring molecular order, and thus reorientational dynamics of 5-DSA spin labels in DPPC were detected using saturation transfer ESR technique. Saturation-transfer ESR not only investigates reorientational dynamics but also provides information on slow motions characterized by correlation times in the range $\tau_c = 10^{-7}$–10^{-3} s (Thomas et al., 1976). Our rationale for using this technique is that the detection of the slow motion of labeled fatty acids may reveal the motional behavioral differences in α-tocopherol- or α-tocotrienol incorporated membranes, which may not be detectable using ^{2}H NMR or conventional ESR measurements where time scales are 3×10^{-6} s (Bloom et al., 1991) and 10^{-11}–10^{-7} s (Marsh, 1981), respectively. Biological significance of the slow time scale in the reorientational dynamics of membrane components has also been suggested (Thomas, 1986).

Indeed, the parameters C′/C and L″/L measured from saturation transfer ESR spectra reveal some differences. The parameters yield information on the reorientational dynamics, respectively, of the motion of 5-DSA around the long molecular axis and of the wobbling motion of the long axis itself (Marsh, 1980; Severcan & Cannistraro, 1990). They exhibit the same qualitative trend with both forms of the vitamin, indicating mobility is increased in the gel phase and decreased in the liquid-crystalline phase. The ratio of correlation times $\tau_c(L''/L)/\tau_c(C'/C)$, however, identifies a distinction in the slow motions which is normally hidden by the detection of molecular motions at faster time scales. Specifically, our results imply that α-tocotrienol causes more anisotropic rotational diffusion of the 5-DSA spin label than does α-tocopherol in the temperature range examined (5–60 °C).

The present study demonstrates that (1) α-tocotrienol is more potent in scavenging peroxyl radicals in liposomes than α-tocopherol; (2) α-tocotrienol and α-tocopherol affect membrane order to similar extents, reducing order in gel phase and increasing order in liquid-crystalline phase; (3) ^{2}H NMR monitors a slightly greater increase in order in the liquid-crystalline phase with α-tocopherol and, since the difference is manifest in the C10–13 region of the phospholipid chain, may suggest different depths of incorporation for the two vitamin E constituents; and (4) different effects on membrane reorientational dynamics are detected by saturation-transfer ESR for α-tocotrienol and α-tocopherol, implying motion becomes more anisotropic in the presence of α-tocotrienol. It is tempting to propose that the differences in membrane reorientational dynamics and ordering identified correlate with the greater antioxidant activity of α-tocotrienol. More work, particularly to determine the physiological relevance of our results which used high vitamin E to lipid ratios in a saturated membrane, is clearly needed to establish a precise structure–function relationship. Development of effective antioxidants is important as oxidative stress has been implicated in the pathogenesis of various diseases (Halliwell & Gutteridge, 1988; Packer, 1992), and understanding of the structural consequences on antioxidant activities should contribute in antioxidant design (Suzuki et al., 1993).

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