Effect of α-Tocopherol on Lipid Peroxidation and Acyl Chain Mobility of Liver Microsomes from Vitamin E-Difficient Rat

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Summary Effects of α-tocopherol on lipid peroxidation and membrane fluidity were studied in liver microsomes from vitamin E (α-tocopherol)-deficient rats using NADPH as a substrate. Microsomes containing various contents of α-tocopherol were prepared by incubation with various concentrations of α-tocopherol in ethanol solution. NADPH-dependent lipid peroxidation decreased the content of polyunsaturated fatty acids, arachidonic acid and 4, 7, 10, 13, 16, 19-docosahexaenoic acid. The treatment with α-tocopherol before peroxidation reduced the production of lipid peroxides and the change in fatty acid composition even at the lowest content of α-tocopherol dealt with in this experiment, 0.2 molar fraction, while addition of α-tocopherol after peroxidation resulted in a slight inhibition of peroxide production and small alteration in fatty acid composition.

By an ESR measurement using stearate spin probe, the α-tocopherol incorporated into microsomes did not alter the acyl chain mobility up to 0.2 molar fraction but reduced the mobility above 0.2 molar fraction. The acyl chain mobility was markedly decreased by lipid peroxidation. The decrease of membrane fluidity was repressed in microsomes treated with α-tocopherol before peroxidation, but was not repressed in microsomes treated after peroxidation.

The experiment using artificial membranes of egg yolk phosphatidylcholine and rat liver phosphatidylcholine revealed that the effect of α-tocopherol on membrane fluidity depends on the fatty acid composition of phospholipid, especially the content of arachidonic acid. On the other hand, the mobility of the fatty acyl chain was not affected by spermine at concentrations which could inhibit lipid peroxidation. These results suggest that the inhibitory effect of α-tocopherol on lipid peroxidation is due to antioxidant activity rather than the indirect effect of membrane stabilization.

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Vitamin E was found to be an anti-infertility factor for the mouse in 1922. The most active vitamin E in nature is d-α-tocopherol, and it is essential in the nutrition of various vertebrates in which its absence is associated with infertility, degenerative changes in muscle or vascular abnormalities. However, the precise mechanism of physiological functions of vitamin E including its protecting action in lipid peroxide production is still unknown. In general, vitamin E is considered to act physiologically as an antioxidant because the effect of vitamin E deficiency is reversed by addition of antioxidant (1, 2). However, there are some phenomena which cannot be properly explained by the antioxidant function (3, 4). Lucy (5) investigated the interactions between vitamin E and polyunsaturated phospholipids, thereby proposing the membrane-stabilizing effect of vitamin E (6). There is also a report that tocopheryl acetate, which does not have antioxidant potency, protects membranes from oxidative damage (7).

There have been several reports concerning the effects of vitamin E concerning lipid peroxidation in membranes. May and McCay (8, 9) showed that oxidation of NADPH by rat liver microsomes resulted in a reduction of polyunsaturated fatty acids in membranes and a concomitant production of lipid peroxide. Lipid peroxidation induced by FeSO₄ and ascorbate was shown to decrease the fluidity of phospholipid bilayer membranes, using fluorescent probes (10). α-Tocopheryl acetate was found to decrease the fatty acyl chain mobility of dipalmitoylphosphatidylcholine membranes (11). In human platelet membranes, fluidity was changed by the addition of α-tocopherol (12). And Fukuzawa et al. have extensively examined the antioxidant effect of α-tocopherol using model membrane systems (13).

In this communication, using microsomes prepared from vitamin E-deficient rat liver, the authors examined the change in fatty acid composition of membrane lipid induced by lipid peroxidation, and its effect on the mobility of phospholipid fatty acyl chains, i.e. membrane fluidity. Moreover, the influence of vitamin E on lipid peroxidation and membrane lipid fluidity was investigated. To monitor membrane fluidity, the fatty acid spin probe was used since the fluorescent probe might produce errors due to intensity changes caused by lipid peroxidation (14).

EXPERIMENTAL

Isolation of microsomes. Rats, male Sprague-Dawley, were fed with vitamin E-free food (Eisai Co., Tokyo) for 4 months. The average body weight increased to about 400 g. The rats were exsanguinated by decapitation, and the livers were then removed and sliced. The slices were homogenized in 0.25 M sucrose containing 0.5 mM EDTA and 10 mM Tris-HCl (pH 7.5) with a Teflon-glass homogenizer. The

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homogenate was centrifuged at 100,000 × g for 60 min with a Hitachi 80P centrifuge (Hitachi Co.). The pellet was resuspended in 125 mM Tris-HCl (pH 7.5), homogenized with a Teflon-glass homogenizer and adjusted to 5 mg protein per ml. All steps were carried out at 4°C.

Treatment of microsomes with α-tocopherol. D-α-Tocopherol (purity 100%) was kindly supplied by Eisai Co., Tokyo. α-Tocopherol at various concentrations (0, 4, 10 and 25 mM) in 1 ml ethanol was added to 10 ml of microsomal suspension after 20 min-preincubation at 37°C, followed by further incubation at 37°C for 30 min. The final concentration was 0, 0.36, 0.90 and 2.25 mM, respectively. The microsomes treated with α-tocopherol were washed in the buffer (25 mM Tris-HCl, pH 7.5) three times with centrifugation. The same treatment with α-tocopherol was performed for microsomes exposed to lipid peroxidation.

NADPH-dependent lipid peroxidation in microsomes. Lipid peroxidation was carried out according to the method of May and McCay (9). Tris-HCl buffer (8 ml) containing 6.25 mM glucose-6-phosphate, 5 mM adenosine diphosphate, 15 μM FeCl₂, 12.5 mM nicotinamide and 375 μM NADPH was preincubated at 37°C for 10 min, and 1 unit (20 μl) glucose-6-phosphate dehydrogenase and 2 ml of microsomal suspension were subsequently added to the solution. The mixed solution was then incubated at 37°C for 30 min and subsequently cooled in an ice bath. In the case of α-tocopherol treatment after peroxidation, the mixture was further incubated at 37°C for 15 min with α-tocopherol at various concentrations. In order to terminate the lipid peroxidation and also to remove the unincorporated α-tocopherol, all samples were washed twice in the Tris-HCl buffer with centrifugation.

Assay of lipid peroxides. The content of lipid peroxides was measured according to the method of Masugi and Nakamura (15). The following reagents were added to 1 ml of the reaction mixture sequentially: 0.2 ml of 8.1% sodium dodecyl sulfate, 2 ml of 0.1 N HCl, 0.3 ml of 10% phosphotungstate and 1 ml of 0.8% thiobarbituric acid. The mixture was heated in boiling water for 45 min, and the product of thiobarbituric acid was extracted into 5 ml of n-butanol. The production of lipid peroxides was calculated from absorbance at 532 nm using tetraethoxypropane as standard.

Assay of α-tocopherol. Two milliliters of ethanol, 100 mg of ascorbate in solution (0.2 ml) and 0.2 ml of KOH were added to the reaction mixture, which was then heated at 100°C for 10 min, cooled in an ice bath, and extracted twice with 5 ml of n-hexane. The extract was concentrated to 0.5–0.1 ml under an N₂ stream, to which was added 1 ml of 1% digitonin in 50% ethanol. It was incubated for 30 min at 25°C and then centrifuged. The extraction from the pellet was repeated and the extracts were combined. The content of α-tocopherol was determined by gas-liquid chromatographic analysis using α-tocopheryl propionate as internal standard. The level of recovery by this method was examined using a radioisotope, d-5-methyl-[¹⁴C]α-tocopherol, and was more than 85%.

ESR spectroscopy. The stearate spin probe, a N-oxy-4’,4’-dimethylox-a
zolidine derivative of 5-ketostearic acid, was synthesized according to the procedure of Waggoner et al. (16). For spin labeling of microsomes, the rounded bottom of a small test tube was coated with 20 μg of spin probe by the evaporation of solvent. Microsomal suspension (1.5 mg protein per ml) was added into the tube and it was incubated at 37°C for 15 min. Microsomes were collected by centrifugation and used for ESR measurement. The spin-labeled microsomes were placed in a cylindrical quartz tube and their ESR spectra were measured at various temperatures using a commercial X-band spectrometer (JEOL FE-2X) equipped with a variable temperature control. The parallel ($T_{||}$') and perpendicular ($T_{\perp}$') principal values of the hyperfine tensor of an axially symmetrical spin Hamiltonian were estimated from the ESR spectra, and the order parameter, $S$, was calculated using the relation

$$S = \frac{T_{||'} - T_{\perp'}}{T_{zz} - \frac{1}{3}(T_{xx} + T_{yy})} \cdot \frac{a}{a'},$$

where $T_{zz}$ (32.9 G) and $T_{xx} = T_{yy}$ (5.9 G) are the hyperfine principal values of the nitrooxide radical and $a'/a$' is the polarity correction factor; $a' = (T_{||'} + 2T_{\perp'})/3$ and $a = (T_{xx} + T_{yy} + T_{zz})/3 = 14.9$ G (17). For the experiment on liposomes, egg-yolk phosphatidylcholine (2.5 μmol), which was extracted according to the procedure of Bligh and Dyer (18), and the spin probe (20 μg), were taken into a small test tube with various quantities of α-tocopherol (0, 0.2, 0.4, 0.6, 0.8 and 1.0 μmol). Tris-buffered saline (150 mM NaCl, 50 mM Tris-HCl, pH 7.5) and several glass beads were added to each tube, and liposomes were prepared by vortexing for 2 min above 40°C. Liposomes of rat liver phosphatidylcholine were prepared by the same method as described above. The effect of α-tocopherol on the phase transition of dipalmitoylphosphatidylcholine (DPPC) was examined using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) spin probe according to a previous report (19). In this case, liposomes were prepared in Tris-buffered saline containing 0.4 mM TEMPO spin probe, and the liposomal dispersion was concentrated by centrifugation (35,000 × g; 10 min) to approx. 200 mg/ml for ESR measurements.

**Analysis of fatty acid composition.** The total lipid was extracted from the microsomes by the method of Bligh and Dyer (18). Methyl esters were prepared by the method of Morrison and Smith (20) and analyzed on a model GC-6 gas chromatograph (Shimadzu, Kyoto). The samples were injected into a 200 × 3 cm glass column packed with 15% diethylene glycol succinate supported on Chromosorb W, 80–100 mesh (Gaschrom Kogyo Co., Tokyo). Column temperature was 185°C and the flow rate of carrier gas, N₂, was 60 ml/min. Peaks of individual fatty acids were quantified by comparison of retention times with those of authentic standards.

**Other analytical methods.** Protein concentration was determined by the method of Lowry et al. (21), and lipid phosphorus by the procedure of Bartlett (22), as modified by Marinetti (23).

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RESULTS

α-Tocopherol content of rat liver microsomes

Microsomes were prepared from livers of vitamin E-deficient rats. The α-tocopherol content of microsomes prepared from these rats was 0.0094 as the molar fraction, \((\alpha\text{-tocopherol})/(\alpha\text{-tocopherol}+\text{phospholipid})\). α-Tocopherol was incorporated into microsomes by incubation with ethanol solution of α-tocopherol. The incorporated content of α-tocopherol was proportional not only to the concentration of α-tocopherol but also to the concentration of ethanol in the suspension. To avoid the ethanol effect on membranes, 9.1% was adopted as the final concentration of ethanol in this experiment. The content of incorporated α-tocopherol was 0.19, 0.45 and 0.64 molar fraction after incubation with 0.36 mM, 0.90 mM and 2.25 mM α-tocopherol, respectively. When microsomes were treated with α-tocopherol at these concentrations after the peroxidation, the content of incorporated α-tocopherol was not different from that in microsomes which were not exposed to lipid peroxidation.

NADPH-dependent lipid peroxidation in microsomes

When NADPH was added to the microsomal preparation, NADPH-dependent lipid peroxidation was observed. Figure 1 shows the profile of production of lipid peroxides by incubation in the presence of NADPH for 30 min at 37°C. α-Tocopherol incorporated into membranes reduced the content of lipid peroxide in proportion to its content. However, when microsomes exposed to peroxidation by NADPH were treated with α-tocopherol for 15 min, the content of lipid peroxide was found to decrease only slightly in microsomes with higher α-tocopherol content.

Effect of α-tocopherol on fatty acid composition of microsomal lipids altered by NADPH-dependent peroxidation

Major components which comprised more than 10% of total microsomal fatty acid composition were palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2) and arachidonic acid (C20:4). Oleic acid (C18:1) and 4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6) were minor components. There was a significant difference in fatty acid composition between the normal and the vitamin E-deficient rats. The level of arachidonic acid was markedly increased in vitamin E-deficient rats. In vitamin E-deficient rats, NADPH-dependent lipid peroxidation was examined for microsomes with various contents of α-tocopherol (Table 1). The content of polyunsaturated fatty acids, C20:4 and C22:6, was decreased by NADPH-dependent lipid peroxidation. Microsomes, which had been exposed to α-tocopherol and then subjected to peroxidation, showed changes in fatty acid composition depending on the content of incorporated α-tocopherol. On the other hand, α-tocopherol added after peroxidation caused a slight recovery from the altered composition, though the underlying mechanism remains at present un-
known. In a quantitative estimation, incubation of microsomes with NADPH for 30 min at 37°C reduced the relative content of arachidonic acid to 80% of the control. Microsomes treated with α-tocopherol at various concentrations prior to peroxidation did not show any marked decrease in the level of arachidonic acid nor C22:6, whereas arachidonic acid content in microsomes treated with α-tocopherol after the peroxidation, decreased to 86% of the control.

**Effects of lipid peroxidation and α-tocopherol on microsomal membrane fluidity**

The effect of α-tocopherol on membrane fluidity was examined by ESR spectroscopy using a stearic acid spin probe. The ESR spectra of microsomes labeled with the spin probe showed a typical pattern of anisotropic motion of the probe situated in lipid bilayers (Fig. 2). The order parameter vs. the content of incorporated α-tocopherol is plotted in Fig. 3 at three different temperatures. The order parameter was scarcely affected by α-tocopherol at less than 0.2 molar fraction, implying that α-tocopherol did not exert any effect on the mobility of fatty acyl chains below this concentration. However, when the α-tocopherol content was raised above the level of 0.2 molar fraction, the order parameter increased markedly.
Table 1. Changes of fatty acid composition by peroxidation and effect of α-tocopherol in vitamin E-deficient rat microsomes.

Microsomes were prepared from rat livers and treated with various concentrations of α-tocopherol before and after peroxidation. Lipid peroxidation was carried out by incubation at 37°C for 30 min in the reaction mixture as described in EXPERIMENTAL. Fatty acid composition of total extracted lipids was analyzed by gas-liquid chromatography using authentic standards. The values indicate averages of two experiments. Fatty acid composition of microsomal lipid from normal rat livers is shown as average ± SD of five experiments.

| % Content of total fatty acids | 16:0 | 18:0 | 18:1 | 18:2 | 20:4 | 22:6 |
|-------------------------------|------|------|------|------|------|------|
| Control (37°C, 30 min)        | 16.3 | 31.1 | 5.0  | 11.3 | 31.9 | 4.4  |
| Exposed to peroxidation       | 19.5 | 34.7 | 6.3  | 12.7 | 24.8 | 2.0  |
| Treated with α-tocopherol before peroxidation | 19.3 | 36.8 | 6.2  | 12.4 | 23.3 | 1.8  |
| with ethanol                  | 16.0 | 31.8 | 5.1  | 11.2 | 31.5 | 4.4  |
| with 0.36 mM α-tocopherol     | 16.0 | 31.5 | 4.8  | 11.0 | 31.6 | 5.1  |
| with 2.25 mM α-tocopherol     | 16.8 | 31.6 | 4.9  | 10.8 | 30.7 | 5.2  |
| Treated with α-tocopherol after peroxidation | 18.8 | 34.5 | 6.6  | 13.4 | 23.0 | 3.7  |
| with ethanol                  | 17.4 | 32.6 | 5.9  | 12.6 | 27.9 | 3.5  |
| with 0.36 mM α-tocopherol     | 17.2 | 32.3 | 5.9  | 12.5 | 27.6 | 4.6  |
| with 2.25 mM α-tocopherol     | 17.5 | 32.7 | 5.9  | 12.6 | 27.5 | 3.7  |
| Normal rats (no treatment)    | 20.2 ± 1.5 | 30.1 ± 1.6 | 7.7 ± 0.8 | 13.9 ± 1.5 | 19.1 ± 1.6 | 7.6 ± 1.7 |

at all temperatures examined. This indicated that the trans-gauch isomerization of fatty acyl chains was inhibited by α-tocopherol.

As shown in Fig. 4, lipid peroxidation caused a marked increase in the order parameter, which correlated well with the decrease in C20:4 and C22:6. In general, the decrease in the relative proportion of unsaturated fatty acids leads to an increase in the order parameter (24).

α-Tocopherol (0.2 molar fraction) treatment before peroxidation suppressed the increase in the order parameter almost completely. But addition of α-tocopherol to microsomes exposed to peroxidation resulted in only a slight suppression of the increase in the order parameter. These results are compatible with changes in fatty acid composition; α-tocopherol treatment prior to peroxidation (before treatment) protected polyunsaturated fatty acids from peroxidation, and the α-tocopherol treatment after peroxidation (after treatment) inhibited the further peroxidation of polyunsaturated fatty acids. The curves of the control (Fig. 3) and the α-tocopherol-treated microsomes (Fig. 4) can be superimposed, which is consistent with the fact that there is no difference between the fatty acid compositions of these microsomes. The increase in the order parameter above 0.2 molar fraction is considered to be due
Fig. 2. ESR spectra of stearate spin probe incorporated into rat liver microsomes. Microsomal suspension (1.5 mg protein per ml) was incubated with 20 μg of spin probe at 37°C for 15 min. (a) ESR spectrum of control microsomes at 30°C. (b) ESR spectrum of microsomes at 30°C after the lipid peroxidation at 37°C for 30 min. $2T_{\parallel}$ and $2T_{\perp}$ are shown in the figure.

Fig. 3. Effect of α-tocopherol on order parameter of stearate spin probes in microsomal membranes. Microsomes were prepared from livers of four vitamin E-deficient rats in each experiment. Microsomes were labeled with stearate spin probe. Averages of two experiments are shown in the figure.

to the interaction of α-tocopherol with the lipid bilayer.

Effect of α-tocopherol on fluidity of rat liver and egg-yolk phosphatidylcholine liposomes

In order to examine the effect of α-tocopherol on the physical state of

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membrane lipid bilayers, fluidity was measured by the spin labeling technique for rat liver and egg-yolk phosphatidylycholine liposomes (Fig. 5). The incorporated α-tocopherol increased the order parameter in a concentration-dependent manner above 0.2 molar fraction of α-tocopherol in rat liver phosphatidylycholine liposomes, whereas the increased order parameter of egg-yolk phosphatidylycholine was not changed within this range of α-tocopherol concentration. The fatty acid composition of these phosphatidylycholines is shown in Table 2. There was a marked difference in the arachidonic acid content; rat liver phosphatidylycholine contains 23.6% of arachidonic acid, whereas egg-yolk phosphatidylycholine, less than 1%. The order parameter of rat liver phosphatidylycholine was higher than that of egg-yolk phosphatidylycholine because of the higher content of long-chain fatty acids.
Fig. 5. Effects of \( \alpha \)-tocopherol on order parameter of stearate spin probes in egg-yolk phosphatidylcholine liposomes and in rat liver phosphatidylcholine liposomes. For the preparation of liposomes, phosphatidylcholine and stearate spin probes containing various amounts of \( \alpha \)-tocopherol were vortexed with glass beads above 40\( ^\circ \)C for 2 min. ESR measurements were carried out at 37\( ^\circ \)C. Data are demonstrated as average \( \pm \) SD of three experiments.

Table 2. Fatty acid composition of normal rat liver and egg-yolk phosphatidylcholines.
Fatty acid composition was analyzed by gas-liquid chromatography using authentic standards.

| Phosphatidylcholines | % Content of total fatty acids |
|----------------------|-------------------------------|
|                      | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C20:4 | C22:0 | C22:6 |
| Rat liver            | 20.2  | 19.5  | 6.2   | 13.6  | 23.6  | 0.4   | 5.2   |       |
| Egg yolk             | 34.4  | 1.0   | 15.4  | 35.2  | 13.0  | 1.0   |       |       |

*Effects of \( \alpha \)-tocopherol on phase transition of dipalmitoylphosphatidylcholine*

Phase transition of dipalmitoylphosphatidylcholine containing \( \alpha \)-tocopherol was studied by using TEMPO spin probe. The partition of TEMPO spin probe between aqueous and lipid phases is demonstrated as the TEMPO parameter, \( \beta \), in Fig. 6. The \( \beta \) value is higher above the transition temperature of dipalmitoylphosphatidylcholine, 41\( ^\circ \)C, since spin probes prefer to situate in the liquid crystalline than in crystalline domain (25). The sharp transition, observed at 41\( ^\circ \)C in dipalmitoylphosphatidylcholine, disappeared in dipalmitoylphosphatidylcholine containing the 0.27 molar fraction of \( \alpha \)-tocopherol which underwent a strong perturbation of the intermolecular interaction of dipalmitoylphosphatidylcholine.

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Fig. 6. Effect of α-tocopherol on phase transition of dipalmitoylphosphatidylcholine. Liposomes of (○) dipalmitoylphosphatidylcholine or (●) dipalmitoylphosphatidylcholine containing the 0.27 molar fraction of α-tocopherol were prepared in Tris-buffered saline including 0.4 mM TEMPO spin probe. TEMPO parameter, \( f \), was calculated from the peak heights of magnetic field according to the following equation: 
\[ f = \frac{H}{H + P} \]
where \( H \) is the peak height of ESR signal from TEMPO spin probe in lipid phase and \( P \) is that in aqueous phase.

Table 3. Effect of spermine on lipid peroxidation and fluidity of rat liver microsomes. Spermine (Nakarai Chemicals, Kyoto; guaranteed reagent) was added to the reaction mixture before peroxidation. Other experimental conditions were the same as in the experiments using α-tocopherol. The content of newly formed lipid peroxides (malondialdehyde) was calculated by subtracting the content in the reaction mixture without NADPH and glucose-6-phosphate dehydrogenase. Order parameter values indicate averages of three measurements.

| Spermine concentration, mM | 0 | 1 | 2.5 | 5 | 10 | 20 |
|----------------------------|---|---|-----|---|----|----|
| % Inhibition of lipid peroxidation | 0 | 15.5 | 11.0 | 17.5 | 36.7 | 44.2 |

| Spermine concentration, mM | 0 | 28 | 48 |
|----------------------------|---|----|----|
| Order parameter at 30°C     | 0.612 | 0.612 | 0.606 |
| at 35°C                     | 0.592 | 0.592 | 0.587 |
| at 39°C                     | 0.570 | 0.572 | 0.570 |

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Effect of spermine on lipid peroxidation and membrane fluidity

It has been reported that NADPH-dependent lipid peroxidation was inhibited by spermine (26), and this was observed also in the present study. However, as seen in Table 3 which shows the effects of spermine on membrane fluidity, no change in the order parameter was seen at spermine concentrations up to 48 mM. Spermine at concentrations which exerted an inhibitory effect on lipid peroxidation was found not to affect membrane fluidity, thus suggesting that the membrane lipid fluidity is not associated with prevention of peroxidation.

DISCUSSION

Oxidation of NADPH by liver microsomes, prepared from vitamin E-deficient rats, produced lipid peroxides and reduced the content of polyunsaturated fatty acids in membrane lipids. Both production of lipid peroxides and decrease of polyunsaturated fatty acids were inhibited by treatment with \( \alpha \)-tocopherol. The inhibitory effect of \( \alpha \)-tocopherol was evident in the 0.2 molar fraction which was the lowest concentration of incorporated \( \alpha \)-tocopherol used in this study. The immobilizing effect of \( \alpha \)-tocopherol on the motion of fatty acyl chains was not observed in the 0.2 molar fraction but was in the 0.45 and 0.65 molar fractions. However, such a rigidifying effect would occur at a lower level of \( \alpha \)-tocopherol because the phase transition of dipalmitoylphosphatidylcholine disappeared with the 0.27 molar fraction. Actually, Schmidt et al. (11) observed, by using fatty acid spin probes, the immobilizing effect of \( \alpha \)-tocopheryl acetate at 0.28 molar fraction in dipalmitoylphosphatidylcholine liposomes in the fluid phase. \( \alpha \)-Tocopherol at 0.2 molar fraction, which is higher by far compared to physiological concentrations, did not cause any immobilization of microsomal fatty acyl chains. Therefore, rat liver and egg-yolk phosphatidylcholine liposomes were employed to examine in more detail the immobilizing effect of \( \alpha \)-tocopherol. This effect was observed above and below 0.2 molar fraction of \( \alpha \)-tocopherol content in rat liver and egg-yolk phosphatidylcholine liposomes, respectively. The most plausible explanation for this finding is the different fatty acid composition between two liposomal preparations. For example, arachidonic acid content was 23% in rat liver and 1% egg-yolk phosphatidylcholine. Microsomes used in the present experiments contained 23–33% arachidonic acid and the immobilizing effect of \( \alpha \)-tocopherol was seen at a rather high concentration. Maggio et al. (27) demonstrated that the interaction of \( \alpha \)-tocopherol with phospholipids was dependent on its fatty acid composition and that the molar ratio of tocopherol to diarachidonylphosphatidylcholine ranged from 0.4 to 2.0 for effective interaction.

The relation between the inhibitory effects on lipid peroxidation and the immobilizing effects on the lipid bilayer of \( \alpha \)-tocopherol was demonstrated by measurement of the order parameter after lipid peroxidation of microsomes pretreated with \( \alpha \)-tocopherol. The order parameter was increased by lipid peroxidation in untreated microsomes, while in microsomes containing the 0.2 molar
fraction of \( \alpha \)-tocopherol prior to peroxidation, the order parameter was unchanged by peroxidation. These observations can be explained by changes induced by lipid peroxidation in the content of polyunsaturated fatty acids. However, in microsomes containing \( \alpha \)-tocopherol above 0.2 molar fraction, the immobilizing effect exceeds the inhibitory effect of \( \alpha \)-tocopherol. Therefore, the order parameter increases according to the \( \alpha \)-tocopherol content. Masugi and Nakamura (28) reported that vitamin E did not directly control the activity level of superoxide scavenging systems, such as superoxide dismutase, glutathione peroxidase and catalase. The main purpose of this experiment was to obtain information as to whether \( \alpha \)-tocopherol serves as a scavenger or as a membrane stabilizer to prevent peroxidation. The results have shown that both \( \alpha \)-tocopherol and spermine suppress lipid peroxidation at concentrations which do not affect membrane fluidity.

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