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FLUIDITY, PERMEABILITY AND ANTIOXIDANT BEHAVIOUR OF MODEL MEMBRANES INCORPORATED WITH α-TOCOPHEROL AND VITAMIN E ACETATE

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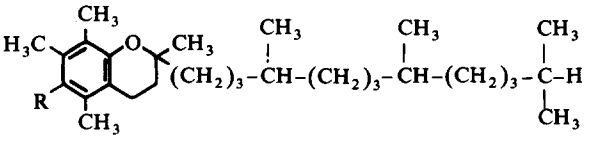
Key words: Model membrane; Membrane fluidity; Membrane permeability; Antioxidant; α-Tocopherol

Magnetic resonance studies reveal a marked difference between the binding of α-tocopherol and that of the corresponding acetate (vitamin E acetate) with dipalmitoylphosphatidylcholine (DPPC) vesicles. This is reflected in differences in the phase-transition curves of the DPPC vesicles incorporated with the two compounds, as well as in the ^{13}C relaxation times and line widths. A model for the incorporation of these molecules in lipid bilayers has been suggested. α-Tocopherol binds strongly with the lipids, possibly through a hydrogen bond formation between the hydroxyl group of the former and one of the oxygen atoms of the latter. The possibility of such a hydrogen bond formation is excluded in vitamin E acetate, which binds loosely through the normal hydrophobic interaction. The model for lipid-vitamin interaction explains the in vitro decomposition of H_2O_2 by α-tocopherol. α-Tocopherol in conjuction with H_2O_2 can also act as a free-radical scavenger in the lipid phase. The incorporation of α-tocopherol and vitamin E acetate in DPPC vesicles enhances the permeability of lipid bilayers for small molecules such as sodium ascorbate.

Introduction

α-Tocopherol (I) is a major component of vitamin E. It is known to function as an efficient inhibitor of lipid peroxidation in vivo [1,2]. The importance of the stereochemical and electronic factors on its effectiveness as an antioxidant has been reported by Burton et al. [3]. Deficiency of vitamin E can lead to the damage of the lipoprotein membranes of cells as well as of organelles such as lysosomes [4]. Hemolytic effects such as hemolysis of human blood cells caused in vitro by oxygen are also known to be counteracted by the presence of α-tocopherol.



R = –OH (α-tocopherol)

II R = –O–C(=O)–CH_3 (vitamin E acetate)

α-Tocopherol is widely used as an anti-sterility agent. The first experiment on its effectiveness as an antisterility vitamin was carried out on rats [5]. This vitamin has been used therapeutically in cases of habitual abortion, muscular dystrophy and cardiac vascular disorders [2,6,7]. The exact molecular mechanism behind such diverse biological functions of α-tocopherol is still unknown. Attempts have been made to understand the interac-

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; TEMPO, 2,2,6,6-tetramethylpiperidine-*N*-oxyl; 5-SASL, 2′,2′-dimethyl-*N*-oxyloxazolidine derivative of 5-ketostearic acid.

tion of α-tocopherol with natural membranes and biological model membranes. For example, α-tocopherol can be exchanged between rat erythrocyte membranes and whole plasma both in vivo and in vitro [8,9]. Nakagawa et al. [10] have calculated the rate of transfer of radioactive α-tocopherol from donor to acceptor liposomes.

An analogue of α-tocopherol is vitamin E acetate (II). It differs from α-tocopherol only in the replacement of the hydroxyl group by an acetate group. Vitamin E acetate has been extensively used in conjuction with α-tocopherol or independently. For example, vitamin E acetate has been used together with α-tocopherol in the treatment of macrocytic, megaloblastic anemia in children [11,12] and hemolytic anemia in premature infants [13].

In the present study, we have incorporated α-tocopherol and vitamin E acetate in lipid bilayers. We have investigated the effect of these two compounds on the structure, fluidity and permeability of model membranes by ESR spin labeling as well as by the ^{13}C-NMR technique. The results indicate a major difference between the behaviour of the two related compounds.

Experimental details

Materials

L-α-Dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma Chemical Company, U.S.A. Spin labels 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) and 2′,2′-dimethyl-*N*-oxyloxazolidine derivatives of 5-ketostearic acid (5-SASL) were purchased from Syva Research Chemicals, U.S.A. Vitamin E acetate and (±)-α-tocopherol were gifts from Malati Chemical Research Centre, India. Hydrogen peroxide and other reagents used were of analytical grade.

Multilamellar dispersions were prepared by the following procedure. Chloroform solutions of lipids were evaporated to dryness with a stream of nitrogen. Residual solvent was removed by drying under vacuum for 2 h and then the appropriate amount of 10 mM phosphate buffer (pH 7.1) was added to the lipids. The system was allowed to equilibrate for 30 min before vortexing and sonication. The lipid-to-vitamin molar ratio used was 5 : 1. The spin label TEMPO was then added to the dispersion. On the other hand the fatty acid spin label was incorporated by adding the required amount of the label to the chloroform solutions of lipids. The initial lipid-to-spin label ratio was chosen as 100 : 1 in phase-transition studies and 50 : 1 in permeability experiments.

Unilamellar vesicles were obtained by sonication of aqueous dispersions using sonifer B-30 (Branson Sonic Power Co.) at a duty cycle of 50% till the dispersions appeared optically clear. It took 40–60 min sonication at 45°C to obtain a clear dispersion. It has been reported [14] that vesicles under such conditions have a diameter of about 250 Å.

Methods

ESR experiments were carried out on an X-band ESR spectrometer with a Varian 12-inch magnet and associated field dial with a VFR 2501 magnet power supply together with a Varian V-45601 100-kHz field modulation and detection unit. Temperatures were controlled to an accuracy of ±1°C using a Varian V-4540 unit. Measurement of the sample temperature was carried out using a copper-constantan thermocouple kept in contact with the sample capillary.

^{13}C spectra were recorded using a Bruker WH 270 spectrometer interfaced with a Nicolet BNC-12 computer. At this field the ^{13}C resonance frequency is 67.89 MHz. In all these experiments a pulse width of 23 μs and repetition time of 10 s were used. Protons were decoupled using broad band noise decoupling. Spin-lattice relaxation times were measured by the inversion recovery method employing ($180° < \tau < 90°$) pulse sequence. All these experiments were performed at a temperature of 50 ± 3°C.

Results and Discussions

Results of phase-transition studies

The phase-transition behaviour of lipid dispersions with and without α-tocopherol has been studied using TEMPO partitioning [15]. This particular spin label has the characteristic property of partitioning between the fluid portion of lipid bilayers and the water phase. The extent of partitioning depends upon the amount of fluid lipids (lipids in the liquid crystalline phase) in the sam-

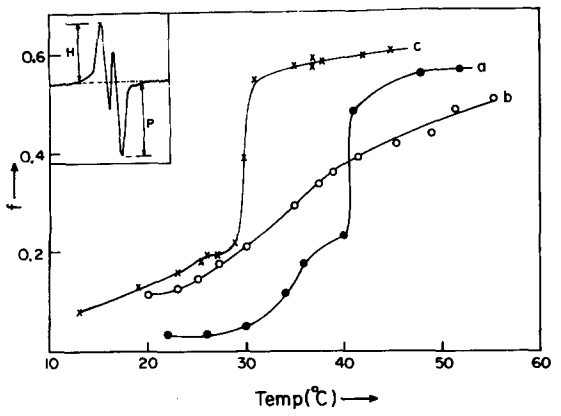


Fig. 1. Spectral parameter $f = H/(H + P)$, as a function of temperature (with H and P as defined in inset; the high-field component of the three-line spectrum splits into a doublet corresponding to TEMPO in lipid (H) and water phase (P)). a (●), DPPC (100 mM); b, (○), DPPC (100 mM)+ α-tocopherol (20 mM); c (x), DPPC (100 mM)+vitamin E acetate (20 mM).

ple. The ESR spectrum of the spin label consists of a three-line pattern due to the hyperfine interaction A of the electron spin with ^{14}N ($I = 1$). The A and the G values of TEMPO are solvent-dependent and this leads to a splitting of the high-field line (Fig. 1 inset). The partition coefficient can be measured by the parameter f defined as $H/(H + P)$, where H is the intensity of the resonance arising from the TEMPO dissolved in the fluid lipid phase and P is that of TEMPO in aqueous phase. Use of this parameter is advantageous as it is not affected by factors such as decomposition of reduction of spin labels, settling of the sample or any other factors which affect spectrometer sensitivity. Parameter f undergoes a gradual increase with increasing temperature and the abrupt large increase corresponds to the gel-to-liquid-crystalline phase transition. In the case of DPPC the main transition is around 41°C, corresponding to the melting of the lipid hydrocarbon chains. There is a pretransition at 35°C which is associated with tilting of the hydrocarbon chains with respect to the plane of the bilayer [16]. Fig. 1 depicts the characteristic phase-transition curve for (a) pure DPPC, (b) DPPC incorporated with α-tocopherol and (c) DPPC incorporated with vitamin E acetate. The characteristic features to the phase transition of pure DPPC dispersions are seen to be more or less retained in the presence of vitamin E acetate. The main phase transition in this case occurs at about 28°C and the pretransition is not as prominent as in the case of pure DPPC bilayers. Schmidt et al. [17] have observed a similar effect. However, when α-tocopherol is incorporated in lipid bilayers the TEMPO partition parameter shows a continuous slow increase with increasing temperature, resulting in a broad melting curve. The sigmoidal behaviour observed for pure DPPC is lost.

Since both α-tocopherol and vitamin E acetate have short polar groups and long hydrocarbon chains, they are likely to form lamellar structures. To check whether water dispersions of pure vitamins show phase-transition characteristics, we have carried out ESR studies of the partitioning of TEMPO in such system as a function of temperature. We observe in such cases, an f value of the order of 0.04 which remains unchanged up to 60°C. This observation implies that the long chains of these compounds do not undergo phase changes over this range of temperature and that a major fraction of spin label remains in the aqueous phase.

Results of ^{13}C-NMR studies

^{13}C-NMR (chemical shifts and relaxation times T_1 and T_2) provide further information on the nature of interaction of vitamin E acetate and α-tocopherol with lipid bilayer vesicles. In C^2HCl_3 solutions, proton decoupled ^{13}C-NMR spectra of both the compounds are characterized by sharp, well-resolved lines at 67.89 MHz (Figs. 2(c) and 3(c)). Assignments of ^{13}C-NMR signals have been reported earlier [18] and we agree with these assignments. For sonicated DPPC vesicles (unilamellar membranes obtained as described earlier) in ^2H_2O solutions, the NMR signals are relatively sharp at temperatures above T_m (Figs. 2(a) and 3(a)).

As in the case of melting curves, the ^{13}C-NMR results also show that the behaviour of vitamin E acetate and α-tocopherol in vitamin-incorporated lipid vesicles is markedly different. When vitamin E acetate is dissolved in lipid vesicles the signals of ^{13}C belonging to both the lipid and vitamin E acetate remain sharp. There is a uniform upfield change in the ^{13}C chemical shifts of vitamin E acetate when it is incorporated in lipid bilayers. The difference between the chemical shift of a particular carbon of vitamin E acetate incorpo-

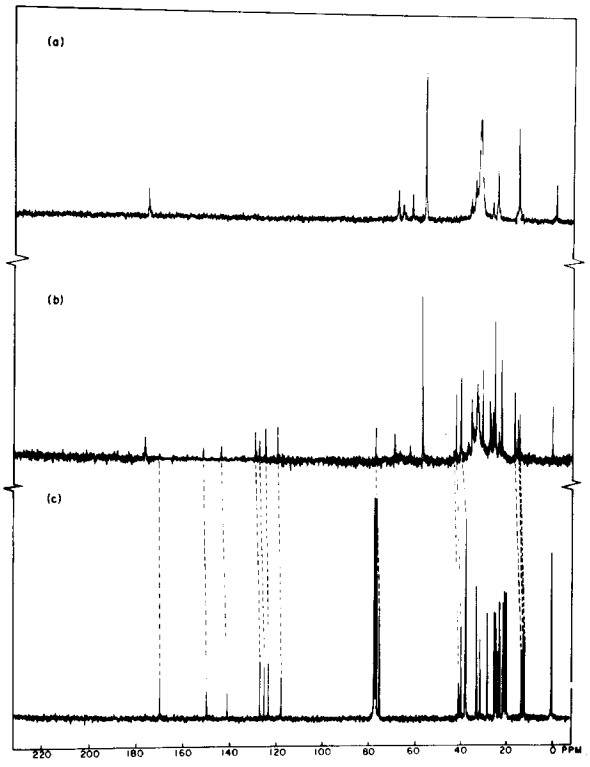


Fig. 2. Proton decoupled ^{13}C-NMR spectra of: (a) 100 mM DPPC dispersed in $^{2}H_2O$ and sonicated, at 50°C; (b) vitamin E acetate (50 mM) incorporated in 100 mM DPPC dispersed in $^{2}H_2O$ and sonicated, at 50°C; (c) vitamin E acetate (50 mM) in $C^{2}HCl_3$.

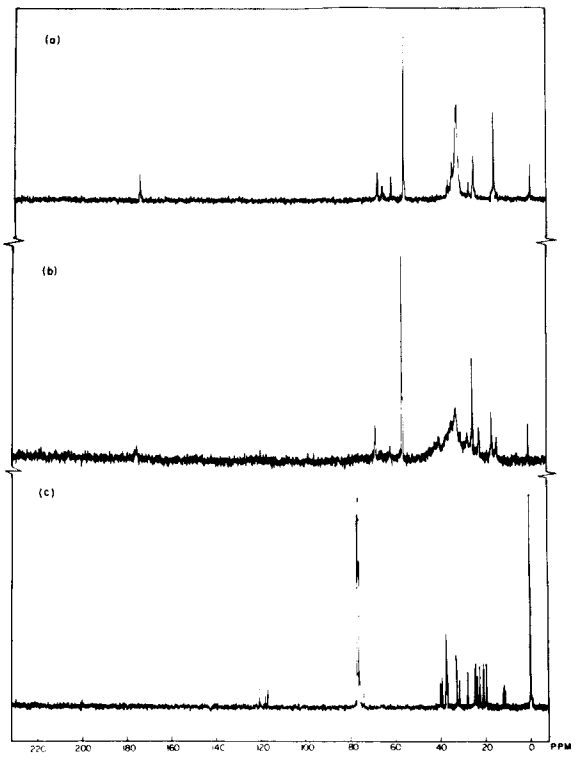


Fig. 3. Proton decoupled ^{13}C-NMR spectrum of: (a) 100 mM DPPC dispersed in $^{2}H_2O$ and sonicated, at 50°C; (b) α-tocopherol (50 mM) incorporated in 100 mM DPPC, dispersed in $^{2}H_2O$ and sonicated, at 50°C; (c) α-tocopherol (50 mM) in $C^{2}HCl_3$.

rated in DPPC bilayer at 50°C and the shift of the same carbon atom when vitamin E acetate is dissolved in $C^{2}HCl_3$ solution lies in the range of 2.2 to 2.5 ppm. This change can be ascribed to the change of environment of vitamin E acetate from $C^{2}HCl_3$ to the more hydrophobic hydrocarbon chains in lipid bilayers.

These findings are further supplemented by ^{13}C spin-lattice relaxation time (T_1) measurements of vitamin E acetate in the free state ($C^{2}HCl_3$ solution) and when bound to the lipid bilayers (Fig. 4). Overall tumbling and segmental motion are the two major factors which contribute to the spin-lattice relaxation time of any nucleus. In proton-decoupled ^{13}C-NMR spectra, the value of NT_1 (where N is the number of protons directly bonded to ^{13}C) is inversely proportional to the rotational correlational time (τ_r). A decrease in NT_1 therefore indicates a decrease in overall rotational motion. Thus NT_1 values are a good measure of the mobility of the nucleus [19]. For vitamin E acetate in $C^{2}HCl_3$ solutions, the values of NT_1 for carbon atoms in the hydrocarbon chains are about 1 s, while those for the methyl carbons are about 3 s. In bound vitamin E acetate, these values are reduced to about 1/3 of the corresponding values in the $C^{2}HCl_3$ solution and are roughly of the order of NT_1 values of ^{13}C of hydrocarbon chains of DPPC bilayers in the liquid crystalline state [20]. This indicates that the motion of hydrocarbon chains in vitamin E acetate is very similar to that of lipid chains in liquid crystalline vesicles. Thus, we can conclude that vitamin E acetate is bound to the lipid bilayer by hydrophobic forces and that at temperatures above T_m it exhibits motions similar to the motion of hydrocarbon chains in phospholipids under similar conditions.

In marked contrast to the behaviour of vitamin

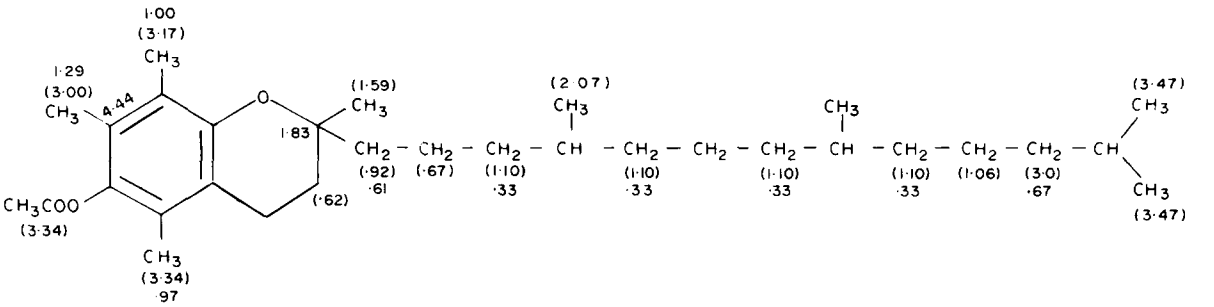


Fig. 4. ^{13}C spin-lattice relaxation time (NT_1) of vitamin E acetate (in seconds) incorporated in lipid bilayers. Numbers in parenthesis refer to NT_1 values of vitamin E acetate in C^2HCl_3, and those outside to NT_1 values for vitamin E acetate bound to lipid vesicles, at 50°C.

E acetate, the ^{13}C-NMR parameters for α-tocopherol are significantly different. For α-tocopherol incorporated in lipid bilayer vesicles, the ^{13}C resonances of both α-tocopherol and DPPC at 50°C are very broad. It is not possible to measure either the chemical shift changes or the spin-lattice relaxation times for these broad signals. However, estimates of spin-spin relaxation times from the line widths indicate that T_2 values are of the order of 100 ms. The lower values of T_2 indicate that the α-tocopherol molecule loses its mobility and becomes strongly bound to the lipid bilayers which results in the loss of motional freedom of α-tocopherol. There is a simultaneous loss in the mobility of lipids which is reflected in the broadening of resonances from DPPC. Evidently, the intermolecular interaction between lipid and α-tocopherol is significantly stronger when compared to the lipid-lipid and lipid-vitamin E acetate interactions.

Vitamin-membrane interaction

From the NMR studies we conclude that the hydrocarbon chain of vitamin E acetate is packed in a manner similar to the loose packing observed for lipids in the liquid crystalline phase of bilayer. By X-ray scattering it has been reported that the hydrocarbon chains in liquid crystalline lipid bilayers are at distances of about 4.7 Å from each other [21]. A model based on a similar packing arrangement for vitamin E acetate is shown in Fig. 5(a). It is observed that vitamin E acetate can be easily incorporated in lipid bilayers with a distance of about 4.7 Å between the hydrocarbon chains of lipids and vitamin E acetate. The aromatic moiety is shown near the lipid-water interface in order to keep the acetate group in the water phase. The interaction between vitamin E acetate and lipid is therefore hydrophobic in nature.

An extension of this model to the binding of α-tocopherol with lipid can be obtained simply by the formation of a hydrogen bond with either of the four oxygens of the phosphate group (Fig. 5(b)) or with the oxygen atoms in the glycerol moiety. Thus, the interaction between α-tocopherol and lipids involves both hydrophobic and hydro-

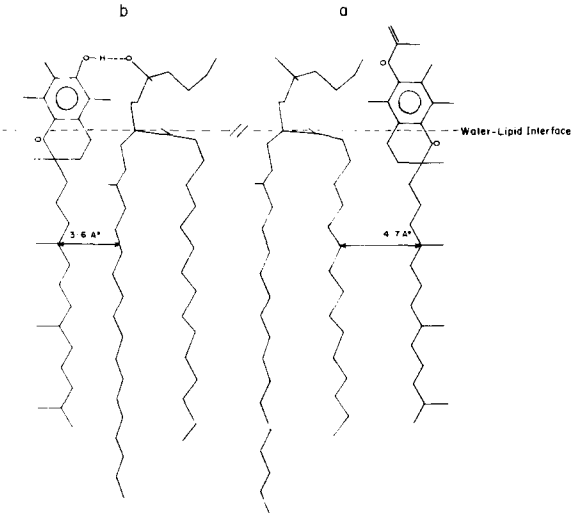


Fig. 5. Model for the interaction of (a) vitamin E acetate with DPPC, (b) α-tocopherol with DPPC. The hydroxyl group of the aromatic ring in (b) in capable of forming hydrogen bonds with oxygen atoms in DPPC and/or with water molecule at the water-lipid interface.

gen-bonding type interactions. It is more likely that the hydrogen bond occurs with one of the two negatively charged oxygens of the phosphate group. The hydrogen bond brings the α-tocopherol molecule closer to the lipid molecule, changing the inter-chain distance to 3.6 Å. These results can be further substantiated by performing interaction energy calculations, which are presently in progress.

It may be pointed out here that the ESR spin labeling results for α-tocopherol are very similar to those with the cholesterol [22]. The phase transition of lipids in the presence of cholesterol shows a broad melting curve devoid of the sigmoidal nature found in the case of pure phosphatidylcholine. Binding of cholesterol with a model membrane system has been studied by various physical techniques such as infrared X-ray and neutron diffraction. X-ray [23] and neutron diffraction [24] studies suggest that the hydroxyl group of cholesterol is located near the lipid-water interface. Infrared spectroscopic results [25] report that the hydroxyl group of cholesterol positions itself in the polar region of the lipids. Besides these studies there are other reports suggesting the formation of a hydrogen bond between cholesterol and phospholipid molecules. The hydrogen bond is formed between the hydroxyl group of cholesterol and with an oxygen atom either in the phosphate group [26,27] or in the ester carbonyl group of phospholipids [28–32]. These results provide further support for our model suggested above for binding of α-tocopherol with model membranes.

The model suggested above for the incorporation of α-tocopherol in the lipid bilayers suggests as interesting mechanism in explanation of the antioxidant properties of α-tocopherol. In this model, α-tocopherol is bound to the lipid bilayers in a fashion so as to position the reducing hydroxyl group in the water phase. This hydroxyl proton will be in dynamic equilibrium between the phosphate oxygens and the interfacial water. Any oxidizing agent approaching the lipid surface would then find a pool of reducing protons and will be reduced before it diffuses into the hydrophobic core of the membrane.

Permeability across model membranes

One of the important properties of biological membranes is the permeability of lipid bilayers [33]. In the liquid crystalline phase, lipid vesicles are able to transport small molecules and ions (passive transport) from the inner to the outer water phase and vice versa. The transport is slowed down considerably as the lipid fluidity changes from liquid crystalline to gel phase. For example, the incorporation of cholesterol is known to decrease the permeability of phospholipid bilayers to Na^+, K^+, Cl^- and glucose [34]. In view of the fact that vitamin E acetate and α-tocopherol have very different modes of binding to lipid bilayers, it is of some importance to investigate how such binding changes the permeability of DPPC vesicles.

For permeability experiments we have used 5-SASL. This spin label dissolves entirely in the lipid phase of the water-lipid system, and, when incorporated in the vesicles, the label gets dispersed both on the inside and the outside monolayer of the vesicles (Fig. 6). If we now introduce a reducing agent such as sodium ascorbate in the water phase, which is on the outer side of the lipid bilayer vesicles, the reducing agent diffuses through the model membrane system and reduces the spin label [35,36]. It may, however, be pointed out that, since sodium ascorbate is introduced on the outside of the bilayers, the spin label molecules residing in the outer monolayer are easily accessible and therefore undergo reduction at a faster rate as compared to the inner monolayer.

We have monitored the reduction of the spin

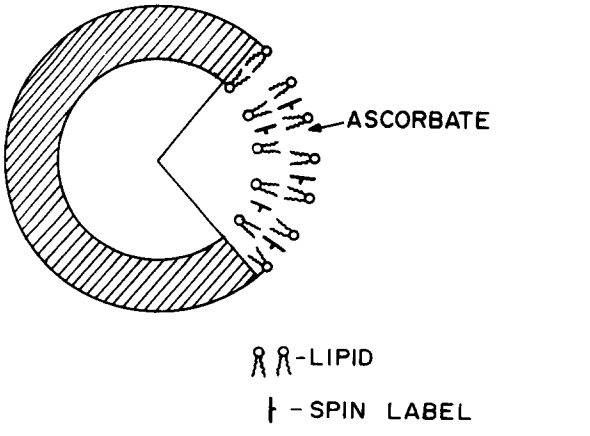


Fig. 6. Permeation of sodium ascorbate through lipid bilayer vesicle incorporated with spin label.

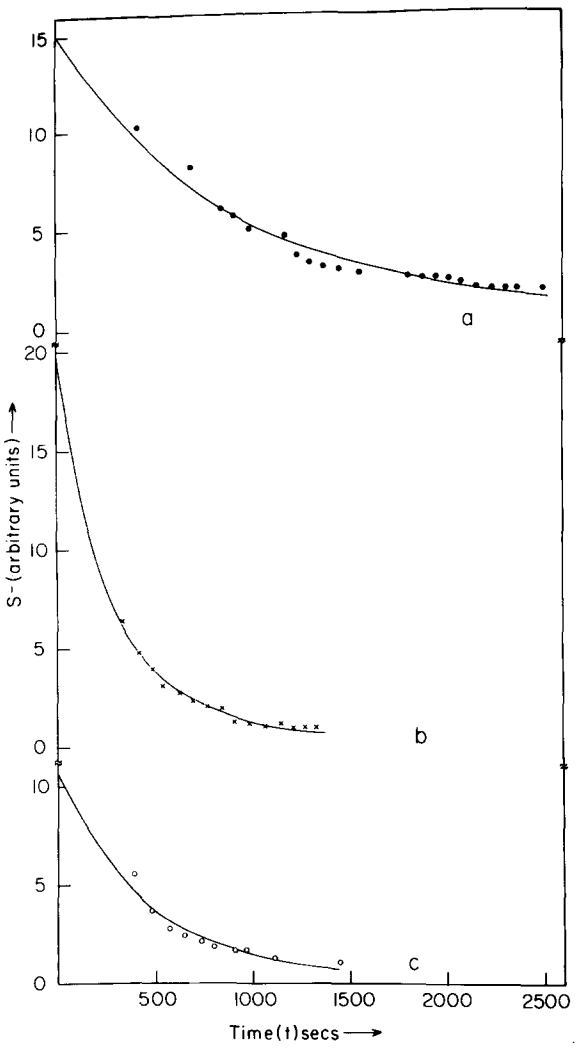


Fig. 7. Signal height $S(t)$ of the ESR spectral line of 2',2'-dimethyl-*N*-oxyloxazolidine derivatives of 5-ketostearic acid spin label (5-SASL) vs. time. The points shown are experimental points through which theoretical curves have been drawn after fitting the data to $S(t) = S_o(0)\ e - k_o t + S_i(0),\ e - k_i t$, where $S_o(0)$ and $S_i(0)$ are respective initial signal heights due to the spin label in the outer and the inner monolayers of sonicated vesicles. a (●), DPPC (100 mM); b (x), DPPC (100 mM) + vitamin E acetate (20 mM); c (○), DPPC (100 mM) + α-tocopherol (20 mM).

label through changes in the signal height of the low-field line in ESR spectrum. We note from Fig. 7 that the ESR signal height decreases with time. Let the rate constant for the reduction of the spin labels present in the outer and inner monolayers to k_o and k_i, respectively. The number of spin labels in the outer and inner monolayers of the vesicle is directly related to the respective surface areas, $4\pi r_o^2$ and $4\pi r_i^2$, where r_o and r_i are the radii of the outer and inner monolayers, respectively. The ESR signal height due to spin label distributed in the outer (S_o) and in the inner (S_i) monolayers will decrease with different rates. The experimental data can then be fitted to an equation:

$$S(t) = S_o(0)e - k_o t + S_i(0)e - k_i t$$

where $S(t)$ is ESR signal height due to total spin label present at time t, and $S_o(0)$ and $S_i(0)$ are signal height due to the initial concentration of spin labels present in the outer and inner monolayers, respectively. We further note that

$$S(0) = S_o(0) + S_i(0) \text{ and } S_o(0)/S_i(0) = r_o^2/r_i^2$$

Taking into account the fact that the outer diameter of the sonicated vesicles is around 250 Å and the thickness of bilayers is around 50 Å [14], $S_o(0)/S_i(0)$ can be estimated. This leaves three unknowns, $S(0)$, k_o and k_i. These are estimated by least-squares fitting of the data on ESR signal height $S(t)$ as a function of time. The goodness of fit was checked by the χ^2 test [37]. The results of such an analysis are given in Table I in terms of half-lives for the reduction of spin label present in the outer and inner monolayers. It may be pointed out that, for pure DPPC bilayers, half-lives for the reduction of spin labels in the outer and inner layer are significantly different, that is, 7.7 and 22.0 min. In this case the results could not be fitted to a single-exponential decay.

Incorporation of α-tocopherol and vitamin E acetate increases the permeability of model mem-

TABLE I

HALF LIFETIME (MINUTES) FOR THE REDUCTION OF SPIN LABEL INCORPORATED IN LIPID BILAYER IN DIFFERENT SYSTEMS AT 45°C

	DPPC	DPPC + α-tocopherol	DPPC + vitamin E acetate
Outer layer	7.7	4.5	5.7
Inner layer	22.0	7.5	5.9

brane to ascorbate ion. As a result the half-lives for reduction of spin label in these cases are considerably less than in the case of pure lipid bilayers. Moreover, in the presence of α-tocopherol and vitamin E acetate the rates of reduction of the spin label residing in the outer and inner monolayers become nearly of the same order. In fact, in these cases the results can be fitted to a single exponential with one half-life without much change in χ^2. The incorporation of α-tocopherol and vitamin E acetate can lead to formation of pores near the site of their incorporation in the vesicles, which in turn can assist rapid equilibration between ascorbate ions residing outside the vesicles and those entrapped inside the vesicles. Diffusion of the ascorbate ion to the site of spin label can thus taken place simultaneously from outside to inside and vice versa. Cushley and Forrest [38] have observed that the permeability of lanthanide ions across phosphatidylcholine vesicles correlates very well with changes in membrane fluidity when α-tocopherol is incorporated in such systems. Our results on the diffusion of ascorbate ion show a similar trend.

α-Tocopherol as an antioxidant

Hydrogen peroxide is produced by living cells in a variety of processes [39]. In polymorphonuclear leucocytes and in macrophages, H_2O_2 appears to play an important role in killing ingested bacteria [40]. The chemistry involved in these processes is not clearly understood, but the findings indicate the possibility of involvement of free radicals. At cellular sites where H_2O_2 is produced [41] hydroxyl radicals resulting from redox reaction between a transition metal ion and H_2O_2 pose a severe threat unless these sites are protected by enzymatic systems capable of destroying peroxides [41]. However, there is not much evidence regarding either the adequacy of these enzymatic protective systems over the lifetime of the cell or the possibility of the hydroxyl radicals 'leaking out' and producing damage at other less-protected sites.

α-Tocopherol plays a key role in catalyzing the decomposition of H_2O_2 in vivo and thus protecting unsaturated lipid from the process of peroxidation. This function is assisted by the binding of α-tocopherol with the lipid bilayers. From our model (Fig. 5) of the interaction of α-tocopherol with model membranes it is evident that the functional group of α-tocopherol is available to the external aqueous phase and can inhibit the peroxidation of lipid by reacting with H_2O_2. We have therefore followed the decomposition of H_2O_2 in DPPC vesicles incorporated with α-tocopherol.

The investigations have been carried out both in the gel phase (21°C) and in the liquid crystalline phase (52°C) of DPPC. A molar ratio of 5 : 1 for DPPC and α-tocopherol was used and the ratio of α-tocopherol and H_2O_2 was varied from 1 : 1 to 1 : 4. The decomposition of H_2O_2 was followed at different times by drawing a small amount of reaction mixture, chilling it and then estimating the residual H_2O_2 by iodometry. The results are shown in Fig. 8. Under similar conditions, lipid vesicles incorporated with vitamin E acetate do not decompose H_2O_2.

Several important observations can be made from the results of these experiments. First, it is observed that almost 2/3 of H_2O_2 is decomposed in less than 2 min. The remaining fraction undergoes a slow decomposition extending over several hours. The fraction of H_2O_2 which undergoes fast decomposition does not depend on the initial concentrations of H_2O_2. The total amount of H_2O_2 thus decomposed can be several times larger than the equivalent amount of α-tocopherol present in the lipid bilayers. α-Tocopherol is found to be active both in the gel and in the liquid crystalline phase.

It appears from these findings that H_2O_2 partitions at a very fast rate between the bulk water and the polar region of lipid bilayers. The H_2O_2 in the water phase is catalytically decomposed rapidly by α-tocopherol. The remaining 1/3 part of H_2O_2 is unavailable to the sites of α-tocopherol and is thus decomposed rather slowly. The living cells should have an additional protection mechanism provided by the enzymatic systems against the H_2O_2 that enters the lipid phase.

The H_2O_2-α-tocopherol system can also jointly act as a free-radical scavenger in the lipid bilayer vesicles. In order to understand this phenomenon we have done the following experiment.

The free radical 5-SASL was used in this experiment. Changes in the ESR signal height were monitored with time in order to estimate the rate of reduction of the free radical. In control experi-

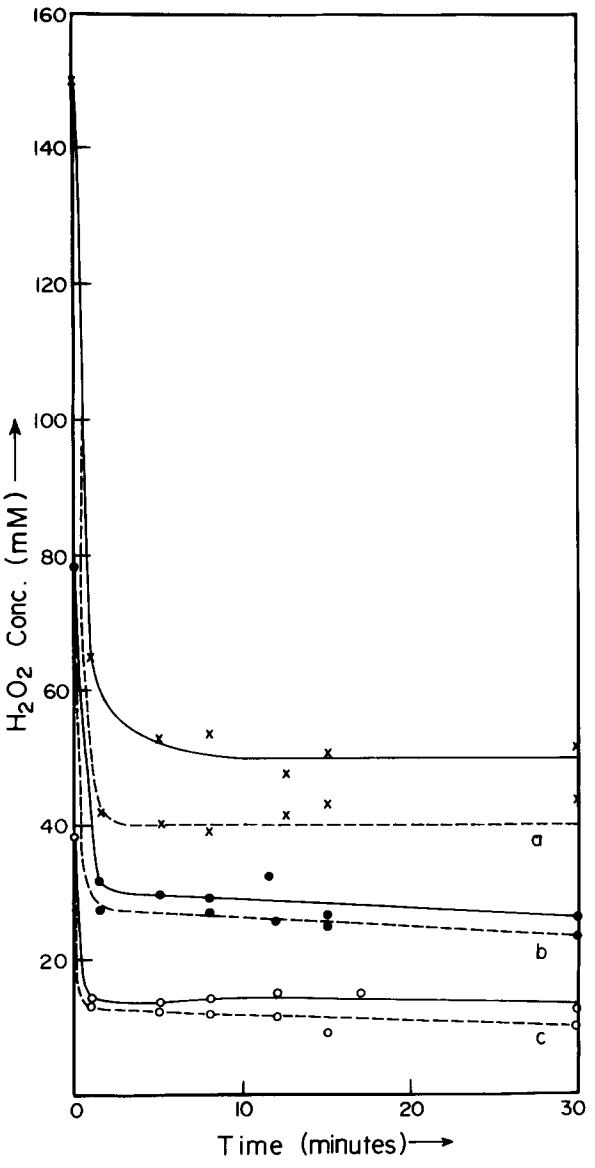


Fig. 8. The kinetics of the decomposition of H_2O_2 at 21°C (solid lines) and 52°C (dotted line) in lipid bilayer vesicles incorporated with α-tocopherol. Concentration of DPPC 195 mM and α-tocopherol 39 mM in all experiments. Initial H_2O_2 concentrations were: a (x) 150 mM; b (●) 78 mM; and c (○) 39 mM.

ments we mixed spin label and H_2O_2 directly. It was observed that the ESR signal height remained unaltered. Spin label was then incorporated in sonicated vesicles of DPPC, and H_2O_2 was added. ESR signal height did not decrease, indicating that H_2O_2 has no effect. The same procedure was repeated with vesicles incorporated with α-tocopherol. The ESR signal height started to decrease and completely disappeared in about 40 min. We therefore conclude that the H_2O_2-α-tocopherol system can act as free-radical scavenger in a model membrane system.

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