# Localization of α-Tocopherol in Membranes[a]

J. C. GOMEZ-FERNANDEZ,[b] J. VILLALAIN,[b]
F. J. ARANDA,[b] A. ORTIZ,[b] V. MICOL,[b]
A. COUTINHO,[c] M. N. BERBERAN-SANTOS,[c]
AND M. J. E. PRIETO[c]

[b] *Departamento de Bioquimica y Biologia Molecular*
*Facultad de Veterinaria*
*Universidad de Murcia*
*E-30071, Murcia, Spain*
[c] *Centro de Quimica Fisica Molecular*
*Instituto Superior Técnico*
*1096 Lisboa Codex, Portugal*

## INTRODUCTION

Tocopherols have been shown to be very important components of biological membranes, where they act mainly as potent antioxidants[1] and contribute to membrane stabilization.[2] In order to understand better the molecular mechanism of tocopherols, it is important to study their interaction with membrane components and especially with lipids. In this way the localization and dynamics of tocopherols in membranes can be known. A number of studies have been done, mainly with α-tocopherol reconstituted in phospholipid vesicles, applying a wide variety of physical techniques. We will summarize in this paper our studies using DSC, FT-IR, and fluorescence spectroscopy.

## PERTURBATION OF PHOSPHOLIPID PHASE TRANSITION BY TOCOPHEROLS

The inclusion of increasing concentrations of α-tocopherol in vesicles of fully saturated phospholipids has been shown to progressively broaden the temperature range of the phase transition of the phospholipid, with its onset temperature being

[a] This work was supported in part by Grant PA86-0211 from Dirección General de Investigación Científica y Technológica (Spain) to J. C. G.-F. and by Research Grant 27777 from Instituto Nacional de Investigação Científica (Portugal) to A. C.

lowered and the enthalpy of the gel to liquid-crystalline transition being reduced. This has been found in studies employing differential scanning calorimetry (DSC),[3-8] electron spin resonance (ESR),[9] $^2$H nuclear magnetic resonance ($^2$H NMR),[8] and Fourier transform infrared (FT-IR) spectroscopy.[5]

It is interesting to note that α-tocopheryl acetate, when included in *dipalmitoylphosphatidylcholine* (DPPC), gave the same effect, but less marked, as in the case of α-tocopherol as shown by DSC (FIG. 1). The lowering and broadening of the phase transition induced by α-tocopherol may be expected from molecules that preferentially partition into fluid domains of the bilayer, decreasing the van der Waals interactions between the terminal methyl and methylene groups of the phospholipid hydrocarbon chain.[7]

FT-IR measurements were also employed, examining bands that report on interactions in the acyl chains ($CH_2$ stretching vibration) and in the interfacial region of

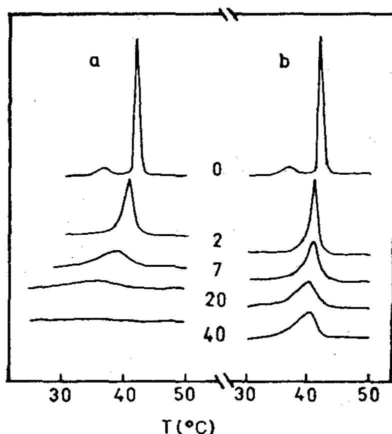


**FIGURE 1.** The DSC calorimetric curves for pure dipalmitoylphosphatidylcholine (DPPC) and DPPC/α-tocopherol systems. Molar percentages of α-tocopherol in DPPC are indicated on the curves. Results correspond to samples containing (a) α-tocopherol and (b) α-tocopheryl acetate. Samples were normalized to the same amount of lipid in each case. Measurements were carried out at 4 K/min in a Perkin-Elmer DSC-4 calorimeter.

the phospholipid (C=O stretching vibration). FIGURE 2 shows that the maximum of the $C_2$ antisymmetric stretching vibration band of pure DPPC is shifted from 2918 $cm^{-1}$ to 2922.4 $cm^{-1}$ during the main phase transition, this shift being associated with the change from all-*trans* to *gauche* conformers.[10-11] The incorporation of increasing concentrations of α-tocopherol produces a progressive broadening of the phase transition and a shift of the onset of this transition to lower temperatures. Both above and below the phospholipid phase transition there is a decrease in *gauche* isomers. α-Tocopheryl acetate broadens the phase transition, however, but does not appreciably affect the proportion of *gauche* conformers at the very high concentration (20 mole-percent) tested.

The perturbation of the membrane interfacial region, produced by α-tocopherol, can be fully appreciated by monitoring the frequency of the C = O stretching band of DPPC (FIG. 3). This band centered at 1735 $cm^{-1}$ and is very broad because of

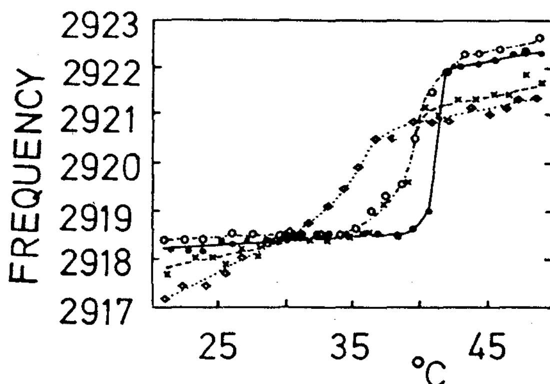


**FIGURE 2.** Temperature dependence of the $CH_2$ antisymmetric band of DPPC. (●——●) Pure DPPC, (○- - - -○) 20 mole-percent of α-tocopheryl acetate, (x---x) 5 mole-percent of α-tocopherol, and (◇---◇) 20 mole-percent of α-tocopherol in DPPC. All infrared spectra have been obtained at 2 $cm^{-1}$ resolution in a Nicolet MX-1 FT-IR spectrometer assisted by a Nicolet 1200-S computer.

the superposition of the bands corresponding to the *sn*-1 and *sn*-2 C = O groups of the fatty acid esters of DPPC, both in dehydrated and in hydrated forms.[12] Two bands can be resolved by different computational techniques, like second-derivative band decomposition, as shown in FIGURE 3, which belong to the dehydrated C = O groups (highest frequency, 1743 $cm^{-1}$ for DPPC at 25°C) and hydrated C = O groups (lower frequency, 1729 $cm^{-1}$ for DPPC at 25°C). As can be observed, the effect of α-tocopherol on the composite band is more pronounced than that of α-tocopheryl acetate. α-Tocopherol induces a decrease in the frequency of the maximum of this band at all temperatures. On the other hand, the effect on the frequency of the

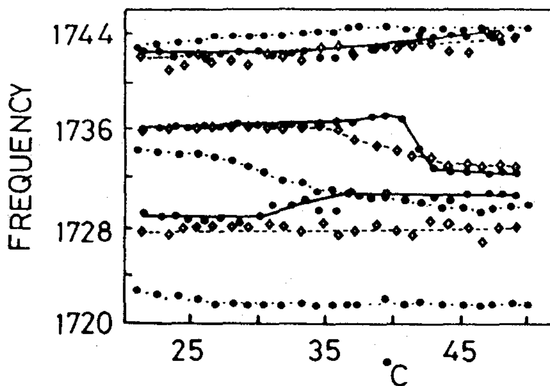


**FIGURE 3.** Temperature dependence of the C=O ester band of DPPC. (○——○) Pure DPPC, (◇---◇) 20 mole-percent of α-tocopheryl acetate, and (●- - - -●) 20 mole-percent of α-tocopherol in DPPC. Upper and lower traces show the *sn*-1 and *sn*-2 components, respectively, and medium traces show the maximum frequency of the composite band.

dehydrated component is small, but, however, it is significant on the hydrated component. α-Tocopherol induced a decrease in frequency of the second component of about 7 $cm^{-1}$, whereas α-tocopheryl acetate induced a decrease of 1-2 $cm^{-1}$ only.

The results shown above indicate that the effect of α-tocopherol is found both in the acyl chains and in the interfacial region of the phospholipid. This conclusion is compatible with the model proposed by Perly *et al.*,[13] where α-tocopherol is situated in both monolayers in an arrangement of phytyl tail to phytyl tail, with the phenolic hydroxyl group located in the lipid/water interfacial region of the membrane. The strong effect of α-tocopherol on the structure of the lipid/water interface of the membrane might be thought to be due to the formation of hydrogen bonding between the α-tocopherol hydroxyl group and the polar part of DPPC, as suggested by Srivastava *et al.*[9] We have not observed significant changes in the phosphate stretching band of DPPC induced by the presence of α-tocopherol; hence it seems that the hydroxyl group of α-tocopherol is hydrogen bonded to a C = O group of DPPC. It is clear that the hydroxyl group of α-tocopherol allows this molecule to be positioned in the bilayer in such a position that the van der Waals interactions with the acyl chains of the phospholipids can be maximized. Because α-tocopheryl acetate has this group blocked by the acetyl replacement, it may not give such a strong perturbation on the DPPC structure, and it would possibly be located in a more hydrophobic position than that of α-tocopherol. The importance of the hydroxyl group of α-tocopherol in determining its interaction with phospholipids has been also emphasized by Lai *et al.*,[14] working with a hemisuccinate ester of α-tocopherol that has a more remarkable lower effect on the phospholipid phase transition than the free α-tocopherol.

## PREFERENCE OF TOCOPHEROL FOR FLUID DOMAINS IN THE MEMBRANE

The concentration of α-tocopherol in biological membranes is rather low. It has been estimated to be in the range of 0.1 to 1.0 mole-percent (of phospholipid).[6] The existence of domains in the membrane with heterogeneous lipid composition, however, could produce an accumulation of α-tocopherol in particular membrane regions. Lipid domains giving lipid heterogeneity in the lateral plane of the bilayer have been postulated for a number of biological membranes, including animal membranes and a plant plasma membrane.[15] These lipid domains, having different lipid composition, have also different degrees of fluidity.

In addition, the transverse asymmetry of plasma membrane phospholipids is a well-documented fact,[16] and evidence exists for differences in fluidity between bilayer halves of the plasma membrane.[17]

Keeping this in mind, we have investigated whether α-tocopherol has a preference for fluid or solid domains in phospholipid vesicles.[7] DSC measurements are presented in FIGURE 4, showing that when α-tocopherol is present in equimolar mixtures of dimyristoylphosphatidylcholine (DMPC) and distearoylphosphatidylcholine (DSPC), which show monotectic behavior, α-tocopherol preferentially partitions in the fluid phase. Experiments were also designed to investigate whether α-tocopherol has a preference for phosphatidylcholine (PC) or for phosphatidylethanolamine[7] (PE).

The interaction of α-tocopherol with PE is different than what is found with PC. FIGURE 5 shows DSC thermograms of DMPE/α-tocopherol mixtures, where it is

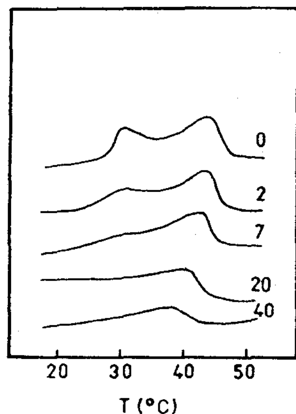


**FIGURE 4.** The DSC calorimetric curves for mixtures of dimyristoylphosphatidylcholine/distearoylphosphatidylcholine (DMPC/DSPC) in a 1:1 molar ratio, containing different amounts of α-tocopherol. Molar-percent contents in α-tocopherol are indicated on the curves.

evident that several peaks are present. It seems that α-tocopherol does not give a good mixing with DMPE, and lateral phase separations occur, probably producing phases with different contents in α-tocopherol and phospholipid, so that the transition temperature will be lower as more α-tocopherol is present in each particular phase. This complex effect may be thought to be due to the perturbation of the intermolecular hydrogen bonds present in vesicles made of this phospholipid.[18] We have confirmed that α-tocopherol can establish hydrogen bonds with DMPE by FT-IR spectroscopy. FIGURE 6 shows the spectra corresponding to the C = O stretching band of pure DMPE: 10 mole-percent of α-tocopheryl acetate in DMPE, and 10 mole-percent of

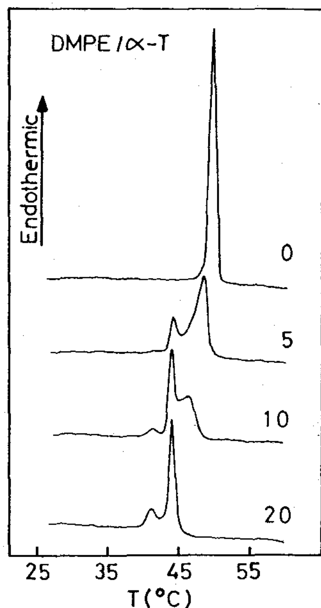


**FIGURE 5.** The DSC calorimetric curves for pure DMPE and systems containing different amounts of α-tocopherol. Molar-percent contents in α-tocopherol are indicated on the curves.

α-tocopherol in DMPE, all of them at 41°C, that is, below the phase transition temperature of pure DMPE (49°C). It can be seen that pure DMPE (FIG. 6A) presents a broad band from which two components may be recovered by spectral deconvolution, as mentioned before.

The inclusion of α-tocopheryl acetate does not produce any significant change in the pattern observed for the pure phospholipid (FIG. 6B). α-Tocopherol, however, produces a significant change in the pattern of this band (FIG. 6C), where it can be seen that the deconvoluted band has three components. The first one appears at 1742 $cm^{-1}$. This high frequency indicates that this group is not engaged in hydrogen bonding, either to water or to phospholipids.[19] The second one is centered at 1734 $cm^{-1}$, and the third component is located at 1718 $cm^{-1}$. Given the low frequency of this component, and by comparison with the spectrum of α-tocopheryl acetate/DMPE (FIG. 6B) where this component is absent, it can be assumed that it corresponds to the C = O ester groups of DMPE, hydrogen-bonded to the hydroxyl group of α-tocopherol.

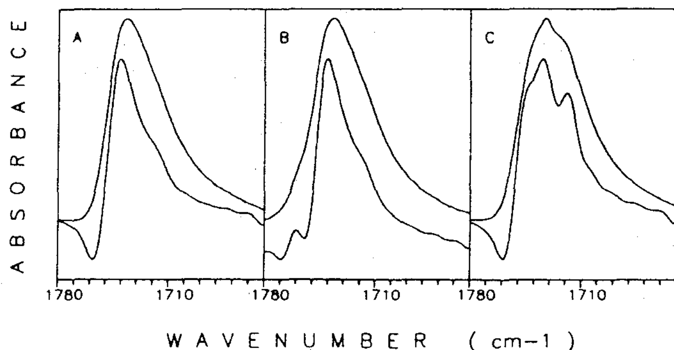


**FIGURE 6.** Infrared spectra of the C=O stretching region of DMPE. (**A**) Pure DMPE, (**B**) 10 mole-percent of α-tocopheryl acetate, and (**C**) 10 mole-percent α-tocopherol in DMPE at 41°C. Lower traces show the corresponding Fourier deconvoluted spectra using Gaussian lines of 20 $cm^{-1}$ full-width at half-height and a resolution enhancement factor of 2.1.

Nevertheless α-tocopherol is shown to preferentially partition into the most fluid domains when it is included in mixtures of PE and PC, independent of whether most dipalmitoyl phosphatidy (ethanolamine (DPPE) fluid domains are richer in PE or in PC.[7] This was shown by DSC experiments in which α-tocopherol was included in vesicles made of equimolar mixtures of either DMPC/dipalmitoylphosphatidylethanolamine (DPPE) or dilauroylphosphatidylethanolamine (DLPE)/DSPC, both mixtures showing monotectic behavior.[7] The preference of α-tocopherol for the more fluid component in these mixtures clearly distinguishes α-tocopherol from cholesterol, which was shown to prefer always PC over PE in phospholipid mixtures.[20]

In view of these observations, it is likely that α-tocopherol will not be homogeneously distributed in the membrane, but rather associated with the most fluid zones. Incidentally, this will cause α-tocopherol to be associated with the most unsaturated fatty acyl chains and hence will facilitate its peroxidation-protecting task.

TABLE 1. Fluorescence Parameters of α-Tocopherol in Solution and Incorporated into Phospholipid Vesicles

| Medium | $\gamma_{max}$ (nm)[a] | $\phi_F$[b] | $\tau_F$ (ns)[c] |
|---|---|---|---|
| Methanol | 316 | 0.43 | ND |
| Ethanol | 317 | 0.34 | 1.8 |
| Acetonitrile | 311 | ND | 1.0 |
| Diethyl ether | 312 | ND | ND |
| Ethyl acetate | 312 | ND | ND |
| *n*-Hexane | 312 | 0.11 | ND |
| Cyclohexane | 312 | 0.16 | 0.8 |
| Dimethylformamide | 311 | ND | ND |
| DPPC (SUV),[d] 25°C | 316 | ND | 1.6 |
| EYL[e] (SUV), 25°C | 316 | ND | 1.7 |

[a] Emission fluorescence when excited at 295 nm, obtained from corrected spectra.
[b] Quantum yields determinated considering $\phi_F$ of naphthalene in ethanol as 0.21.[30]
[c] Fluorescence lifetimes determined by the single photon counting technique.
[d] Small unilamellar vesicles of dipalmitoylphosphatidylcholine.
[e] Egg yolk lecithin.

## LOCALIZATION OF TOCOPHEROL IN PHOSPHOLIPID VESICLES AS SEEN BY ITS INTRINSIC FLUORESCENCE

α-Tocopherol has intrinsic fluorescence, and we have tried to exploit this property inasmuch as this is a very convenient means of directly observing the molecule. First of all, we determined some fluorescent parameters of α-tocopherol. TABLE 1 shows $\lambda_{max}$ of the fluorescence emission spectra, quantum yield ($\phi_F$), and fluorescence lifetime ($\tau_F$), determined for α-tocopherol in a number of solvents and incorporated into phospholipid vesicles. It is interesting that the $\lambda_{max}$ and the $\tau_F$ obtained for α-tocopherol in phospholipid vesicles are similar to those obtained in protic solvents (*e.g.* ethanol). This observation suggests that the chromanol moiety of α-tocopherol should be situated in a polar region of the model membrane, in agreement with previous suggestions.[9,18]

An important point when considering the intrinsic fluorescence of α-tocopherol in the membrane is to know whether all the molecules will be fluorescent or if nonfluorescent aggregates may be formed, as claimed recently.[21] To discern between these two possibilities, we have done the absorption spectra of α-tocopherol in an aprotic organic solvent like *n*-hexane. FIGURE 7 shows that the maximum of the

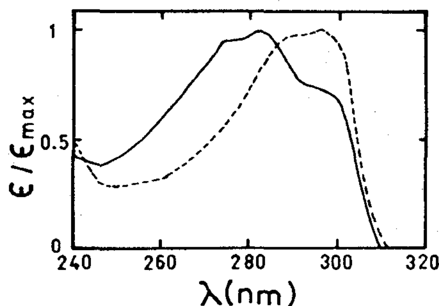


**FIGURE 7.** Absorption spectra of α-tocopherol in *n*-hexane at a concentration of (—) $4.4 \times 10^{-5}$M and (----) $1.42 \times 10^{-4}$ M.

spectrum at low concentration of α-tocopherol in *n*-hexane is at 283 nm (predominance of the monomeric form), but it is shifted to 295 nm at a much higher concentration (predominance of the hydrogen bound dymer). Experiments made with α-tocopherol in phospholipid vesicles showed that α-tocopherol has a maximum near 295 nm within a wide range of concentrations (data not shown), indicating that most α-tocopherol molecules are associated. It could not be discerned from these data whether α-tocopherol molecules are associated, when present in membranes, (presumably through hydrogen bonding), between themselves or with phospholipid or water molecules.

The experiments of FIGURE 8, however, clearly show that although α-tocopherol molecules are associated, the aggregates formed are fluorescent, because linear relationships are found between fluorescent intensity and concentrations in a large range, both in *n*-hexane solution and incorporated into small unilamellar DPPC vesicles at 25°C (up to molar ratios 4:1 DPPC: α-tocopherol). A similar result was obtained with α-tocopherol incorporated into multibilayer vesicles of egg yolk lecithin at 25°C (not shown).

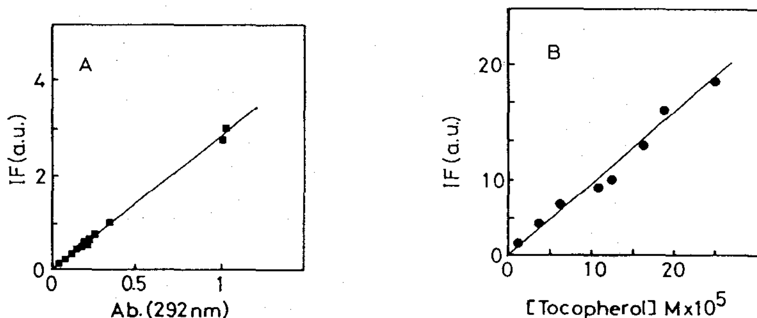


**FIGURE 8.** Fluorescence intensity (in arbitrary units) of α-tocopherol corrected for the inner filter effect versus concentration of α-tocopherol (**A**) in *n*-hexane (concentration expressed as absorbance in *n*-hexane) and (**B**) in small unilamellar vesicles (SUV) made of DPPC at 25°C (lipid concentration $10^{-3}$M).

The localization of α-tocopherol in the bilayer has been also approached through the quenching of its intrinsic fluorescence by membrane probes like 5-doxylstearate (5-NS), which has its nitroxide group at carbon-5, and 16-doxylstearate (16-NS), with the nitroxoide group at carbon-16. These probes have been used before in a number of similar studies designed to study the localization of chromophores in membranes.[22] FIGURE 9 shows that 5-NS quenches α-tocopherol fluorescence much more effectively than 16-NS, as would be expected if the chromanol moiety is located near the lipid/water interface. The quenching process seems to follow a collisional mechanism as shown by the linearity of the Stern-Volmer plots.

Similarly we have also attempted to study the localization of α-tocopherol in egg yolk phosphatidylcholine multilamellar vesicles (MLV) by using a set of n-(9-anthroyloxy) stearic acid (*n*-AS) probes. The explicit distance dependence ($r^{-6}$) of electronic energy transfer (dipolar mechanism) has allowed its application as a spectroscopic ruler for determining distances in biological systems.[23,24] Energy transfer from α-tocopherol (donor), to the fluorescent probes *n*-AS (acceptor), is moderately efficient

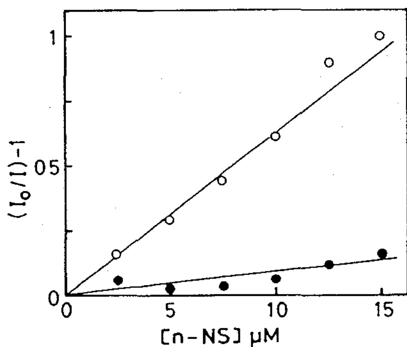


**FIGURE 9.** Stern-Volmer plots of quenching of α-tocopherol fluorescence in DPPC at 50°C by (○) 5-NS and (●) 16-NS. Lipid concentration was kept at 0.25 mM, and DPPC/α-tocopherol molar ratio was 100:1.

with a Förster critical radius, Ro = 14 Å. In egg yolk phosphatidylcholine MLV, different transfer efficiencies were obtained for the family of probes (FIG. 10).

The *n*-AS probes are known to be located at a graded series of depths from the surface, depending on their substitution position (n), in the aliphatic chain.[25] Considering that the Ro value is identical for all acceptors, it can be concluded that the chromophore group of α-tocopherol is situated in the membrane in a region between the 9-anthroyloxy located at carbon-7 and that at carbon-2, the former being the nearest one. This attribution is again compatible with other previous results we have already shown. Furthermore, we have found that acrylamide, which is a water-soluble fluorescence quencher, is a very inefficient quencher of α-tocopherol in fluid membranes (results not shown), acrylamide being an efficient quencher of α-tocopherol in ethanolic solution. This indicates that although α-tocopherol may have its chromanol group relatively close to the polar part of the membrane, it is not sufficiently exposed to allow acrylamide to reach it, acrylamide being known to have a very low capacity of penetration through phospholipid bilayers.[26] Hence the conclusion of these studies on the location of α-tocopherol in phospholipid vesicles is that its hydroxyl group may

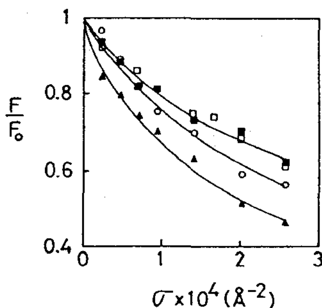


**FIGURE 10.** Relative yield of α-tocopherol fluorescence ($F/F_o$) versus σ (superficial concentration) of (○) 2-AS, (▲) 7-AS, (■) 9-AS, and (□) 12-AS in egg yolk lecithin (EYL) at 25°C. Samples were prepared by combination of chloroform solutions containing EYL and the appropriate amounts of donor and acceptor. After drying, multilamellar vesicles were formed by addition of aqueous buffer and careful mixing. Each point in the plot represents different experimental sets. Measurements were carried out in a Shimadzu RF-640 spectrofluorometer, the excitation and emission wavelengths being 295 and 329 nm, respectively.

be located at the lipid/water interface, forming a hydrogen bond with carbonyl or phosphate groups of phospholipids, and its chromanol moiety lies in a position close to that occupied by 7-AS and 5-NS. The situation of the hydroxyl group of α-tocopherol in the lipid/water interface is of interest in explaining its mechanism of action because any oxidizing agent approaching the membrane surface will find reducing protons. Hence the introduction of those agents in the membrane will be prevented.

## LATERAL DIFFUSION COEFFICIENT OF α-TOCOPHEROL IN PHOSPHOLIPID VESICLES

In order to understand the mechanism of action of α-tocopherol in membranes and how it may be active at very low concentrations, it may be very illustrative to know the lateral diffusion coefficient of this molecule when incorporated into phospholipid vesicles. This can be approached through studies of the quenching of the intrinsic fluorescence of α-tocopherol by 5-NS. This quenching has been shown above to be effective (FIG. 9).

The extent of collisional quenching in a lipid bilayer depends upon the lipid/water partition coefficient and upon the rate of diffusion of the colliding species in the lipid bilayer.[27] Therefore the collisional quenching of fluorescence can be used for calculating the diffusion coefficients for α-tocopherol in lipid vesicles. In a membrane, where quenching occurs only in the lipid phase and where partition with the water phase may be significant, the Stern-Volmer relation for collisional quenching is modified, and the following relation applies:

$$1/\kappa app = \alpha m\ (1/\kappa m - 1/\kappa mP) + 1/\kappa mP$$

where $\kappa app$ is the apparent (measured) bimolecular quenching constant in $M^{-1}s^{-1}$, $\alpha m$ is the fractional volume of the membrane, $\kappa m$ is the bimolecular quenching constant in the membrane phase, and $P$ is the partition coefficient, in units of (moles of quencher per liter of phospholipid)/(moles of quencher per liter of water). The partition coefficient is also expressed as a mole fractional ratio, that is, (moles of quencher per mole of phospholipid)/(moles of quencher per mole of water). A plot of $1/\kappa app$ as a function of $\alpha m$ gives a straight line, with $1/\kappa mP$ as intercept and $(1/\kappa m - 1/\kappa mP)$ as slope.

FIGURE 11A shows Stern-Volmer plots for the quenching of α-tocopherol fluorescence when incorporated into egg yolk lecithin (EYL) MLV at 25°C, by 5-NS, at different lipid concentrations, keeping the molar ratio EYL/α-tocopherol as 100:1. From these plots, $\kappa app$ values were calculated, and they were used for the plot shown in FIGURE 11B. From the last plot (FIG. 11B), $\kappa m$ was calculated to be $3.4 \times 10^9$ $M^{-1}s^{-1}$, and $P$ was found to be $3.2 \times 10^4$ (moles of 5-NS per mole of EYL)/(moles of 5-NS per mole of water).

It is noteworthy that this value for the partition coefficient is very similar to that found by other authors for asolectin bilayers at 25°C,[28] which was $5.9 \times 10^4$ expressed in the same units. In order to calculate the lateral diffusion coefficient of α-tocopherol in the membrane, we have followed the Smoluchowski equation as modified for fluorescence quenching measured in the steady state including transient effects:[29]

$$\kappa_m = 4\pi N' R_{pq}\ \gamma\ (D_p + D_q)(1 + R_{pq}\gamma/\sqrt{(D_p + D_q)\tau o})$$

where $\gamma$ is the quenching efficiency of the fraction of collisional encounters that are effective in quenching; $R_{pq}$ is the sum of the molecular radii of probe plus quencher; $N'$ is Avogadro's number per millimole; $D_p$ and $D_q$ are the diffusion coefficients of the probe and the quencher, respectively, in the membrane, in units of $cm^2s^{-1}$; and $\tau o$ is the fluorescence lifetime of the probe in the absence of quencher.

From the van der Waals radii we estimate a molecular radius of 4.25 Å for α-tocopherol and 4.55 Å for 5-NS. We have assumed $\gamma$ to be 1, so that the calculated diffusion coefficient is the smallest possible and would be higher for $\gamma < 1$. See Fato *et al.*[28] for a discussion of the problems that can be found when trying to estimate $\gamma$ for this type of system. $\tau_o$ was estimated to be 1.7 nanoseconds. Finally, we have assumed $D_q$ to be $2.5 \times 10^{-7}$ $cm^2$ according to Fato *et al.*,[28] which found this value for a similar fluid membrane.

All of this leads to a $D_p$ value of $4.8 \times 10^{-6}$ $cm^2s^{-1}$. This means that α-tocopherol has a very high mobility in a fluid bilayer, and hence it will be quite efficient in order

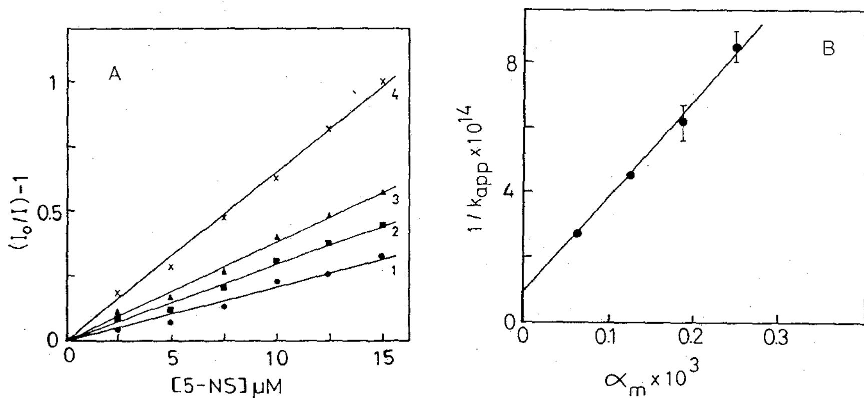


**FIGURE 11. A:** Stern-Volmer plots of quenching of α-tocopherol fluorescence by 5-NS at 25°C in egg yolk lecithin vesicles at different membrane fractional volumes ($\alpha_m$) : (1) $0.25 \times 10^{-3}$; (2) $0.1875 \times 10^{-3}$; (3) $0.125 \times 10^{-3}$; (4) $0.0625 \times 10^{-3}$. The α-tocopherol to phospholipid ratio was kept 1:100 (mol:mol). Experimental procedures are as described in the legend of FIGURE 10. **B:** Plot of $1/\kappa app$ versus membrane fractional volumes ($\alpha_m$) using values from panel **A.** Each point represents the average value ±SE of three different experiments.

to act wherever it is needed. This value is very similar to the one calculated by other authors[28] for ubiquinone-3 ($5.8 \times 10^{-6}$ $cm^2s^{-1}$), which is a molecule very similar in structure to α-tocopherol.

It appears then, that there might be, at least, two mechanisms to compensate for the low concentration of α-tocopherol in the membrane, its preferential partitioning in most of the fluid domains, and its high lateral mobility in the plane of the bilayer.

## CONCLUSIONS

The hydroxyl group of α-tocopherol is essential in determining its effect on phospholipid membranes where it disrupts the lipid/water interface and weakens the van

der Waals interaction between the fatty acyl chains. Hydrogen bonds are established between the hydroxyl group of $\alpha$-tocopherol and a C = O group of DPPC or DMPE. $\alpha$-Tocopherol favorably partitions into the most fluid domains when included in mixtures of phospholipids with monotectic behavior. The study of the intrinsic fluorescence of $\alpha$-tocopherol reveals that its chromophore group is situated in the membrane in a relatively polar position but not readily accessible to the water domain. This is confirmed by resonance energy transfer studies using a series of probes located at graded depths in the membrane.

The lateral diffusion coefficient of $\alpha$-tocopherol in fluid phospholipid vesicles was calculated through quenching of its intrinsic fluorescence by a spin probe. A value of $4.8 \times 10^{-6}$ $cm^2s^{-1}$ was calculated, indicating a very high lateral diffusion of $\alpha$-tocopherol.

## REFERENCES

1. BURTON, G. W., K. H. CHEESEMAN, T. DOBA, K. U. INGOLD & T. F. SLATER. 1983. *In* Biology of Vitamin E (Ciba Foundation Symposium 101): 4-18. Pitman. London.
2. TAPPEL, A. L. 1972. Ann. N. Y. Acad. Sci. **205:** 12-28.
3. MASSEY, J. B., H. S. SHE & H. J. POWNALL. 1982. Biochem. Biophys. Res. Commun. **106:** 842-847.
4. LAI, M. Z., N. DUZGUNES & F. C. SZOKA. 1985. Biochemistry **24:** 1646-1653.
5. VILLALAIN, J., F. J. ARANDA & J. C. GOMEZ-FERNANDEZ. 1986. Eur. J. Biochem. **158:** 141-147.
6. MCMURCHIE, E. J. & G. H. MCINTOSH. 1986. J. Nutr. Sci. Vitaminol. **32:** 551-558.
7. ORTIZ, A., F. J. ARANDA & J. C. GOMEZ-FERNANDEZ. 1987. Biochim. Biophys. Acta **898:** 214-222.
8. WASSALL, S. R., J. L. THEWALT, L. WONG, H. GORRISEN & R. J. CUSHLEY. 1986. Biochemistry **25:** 319-326.
9. SRIVASTAVA, S., R. S. PHADKE, G. GOUIL & C. N. R. RAO. 1983. Biochim. Biophys. Acta **734:** 353-362.
10. ASHER, I. M. & I. W. LEVIN. 1977. Biochim. Biophys. Acta **468:** 63-72.
11. CAMERON, D. G., H. L. CASAL & H. H. MANTSCH. 1980. Biochemistry **19:** 3665-3672.
12. BLUME, A., W. HUBNER & G. MESSNER. 1988. Biochemistry **27:** 8239-8249.
13. PERLY, B., I. C. P. SMITH, L. HUGHES, G. W. BURTON & K. U. INGOLD. 1985. Biochim. Biophys. Acta **734:** 353-362.
14. LAI, M. Z., N. DUZGUNES & F. C. SZOKA. 1985. Biochemistry **24:** 1646-1653.
15. STORCH, J. & A. M. KLEINFELD. 1985. Trends Biochem. Sci. **10:** 418-421.
16. BERGELSON, L. D. & L. I. BARSUKOV. 1977. Science **197:** 224-230.
17. DIPLOCK, A. & J. A. LUCY. 1973. FEBS Lett. **29:** 205-210.
18. HITCHCOCK, P. B., R. MASON & G. G. SHIPLEY. 1975. J. Mol. Biol. **94:** 297-299.
19. WONG, P. T. T. & H. H. MANTSCH. 1988. Chem. Phys. Lipids **46:** 213-224.
20. VAN DIJCK, P. W. M., B. DE KRUIJFF, L. L. M. VAN DEENEN, J. DE GIER & R. A. DEMEL. 1976. Biochim. Biophys. Acta **455:** 576-587.
21. KAGAN, V. E. & P. J. QUINN. 1988. Eur. J. Biochem. **171:** 661-667.
22. BLATT, E. & W. H. SAWYER. 1985. Biochim. Biophys. Acta **822:** 43-62.
23. STRYER, L. 1978. Ann. Rev. Biochem. **47:** 819-846.
24. ENNG, B. K. K. & L. STRYER. 1978. Biochemistry **17:** 5241-5248.
25. THULBORN, K. R. & W. H. SAWYER. 1978. Biochim. Biophys. Acta **511:** 125-140.
26. CHALPIN, D. B. & A. M. KLEINFELD. 1983. Biochim. Biophys. Acta **731:** 465-474.
27. LAKOWICZ, J. R. & D. HOGEN. 1980. Chem. Phys. Lipids **26:** 1-40.
28. FATO, R., M. BATTINO, M. DEGLI ESPOSTI, G. PARENTI CASTELLI & G. LENAZ. 1986. Biochemistry **25:** 3378-3390.
29. UMBERGER, J. Q. & V. K. LAMER. 1945. J. Am. Chem. Soc. **67:** 1099-1109.
30. PARKER, C. A. 1968. *In* Photoluminescence of solutions. 266-267. Elsevier. Amsterdam.