Biochimica et Biophysica Acta, 1105 (1992) 237–244

BBAMEM 75575

Interaction of α-tocopherol with fatty acids in membranes and ethanol

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(Received 23 September 1991)

Key words: α-Tocopherol; Polyunsaturated fatty acid; Fatty acid; Ethanol; Phospholipid bilayer; Membrane

The techniques of fluorescence polarization, ultraviolet light absorbance and fluorescence quenching by acrylamide are used to probe the structural role of α-tocopherol in phospholipid bilayers. Using 1,6-diphenyl-1,3,5-hexatriene (DPH) and a series of (anthroyloxy)stearic acid (AS) fluorescence probes, α-tocopherol is shown to increase fluidity and decrease order of gel state bilayers, and to decrease fluidity and increase order of bilayers in the liquid crystalline state. More complex behavior is noted for bilayers made from mixed acyl chain phosphatidylcholines (PCs) where the *sn*-1 position is saturated and the *sn*-2 position unsaturated compared to bilayers composed of PCs where both acyl chains are either saturated or unsaturated. Complexation between α-tocopherol and either free fatty acids or fatty acids esterified to the *sn*-2 position of PCs is indicated by ultraviolet light absorbance in both organic solution and in lipid bilayers. The strength of the complexes, expressed as interaction constants, are dependent upon the number of acyl chain unsaturations from 0 (stearic acid), to 6 (docosahexaenoic acid). Relation of the strength of these complexes to the degree of acyl chain unsaturation is confirmed by monitoring the fatty acid protection from acrylamide bleaching of α-tocopherol. These experiments suggest that the extent of acrylamide bleaching is related to the extent of association with the fatty acids.

Introduction

α-Tocopherol (vitamin E) has been proposed to support at least two distinct functions in biological membranes; that of an antioxidant [1,2] and as a stabilizing agent for the membrane [3,4]. While the antioxidant role has been well documented, participation of the vitamin in membrane stabilization has been more difficult to define. Interactions between vitamin E and membranes have been followed by fluorescence polarization techniques [5,6], EPR [7–9], NMR [10–12], Fourier transform-infrared spectroscopy [13], ultraviolet spectroscopy [14,15] and differential scanning calorimetry [16–18]. Most of these experiments agree that the bulky chromanol group of α-tocopherol resides near the aqueous interface while the phytyl side chain extends towards the center of the membrane [11,13,19]; the molecule increases order and decreases fluidity in liquid crystalline membranes [5,12,16,20]; and the temperature and enthalpy of the phase transition are reduced in saturated phosphatidylcholine bilayers [12,16–18].

Contradictory reports, however, have appeared. Bisby and Ahmed [21] proposed that the chromanol group lies closer to the aqueous interface of membranes in the gel state than it does with those in the liquid crystal state [21], while Lessard and Fragata [22] reported the vitamin's depth in the membrane is dependent on the osmotic strength of the bathing solution. Cushley et al. [19,23] have suggested that in liquid crystalline bilayers α-tocopherol increases fluidity (by ^{13}C-NMR) but has no effect on membrane order (by EPR), while Veti et al. [24] agreed that the vitamin increases fluidity of human erythrocyte ghosts. There is little agreement on whether α-tocopherol increases or decreases bilayer permeability. α-Tocopherol-induced increases in permeability have been reported for ascorbate [8], water [25] and Pr^{3+} [19,23]. Contrary to these reports, Diplock et al. [26] reported α-tocopherol de-

Abbreviations: AS, (9-anthroyloxy)stearic acid; DHA, docosahexaenoic acid; DMPC, dimyristoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; DSPC, distearoylphosphatidylcholine; PC, phosphatidylcholine; TLC, thin-layer chromatography.

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creases permeability to glucose and chromate while Stillwell and Bryant [27] demonstrated decreases in erythritol and urea permeability caused by the vitamin. Fukuzawa et al. [28] reported an α-tocopherol-induced decrease in glucose permeability for membranes in the liquid crystalline state became a permeability enhancement when the same membranes were put in the gel state.

The nature of interaction between α-tocopherol and fatty acyl chains is also controversial. Diplock and Lucy [26] proposed that the interaction between α-tocopherol and unsaturated fatty acids in membranes was the result of the methyl groups of the isoprenoid side chain of the vitamin intercalating into the z-pockets produced by the unsaturations in the fatty acids. By fluorescence polarization and ^{13}C-NMR, Urano et al. [5,10] have concluded that the α-tocopherol-fatty acid association is through the methyl groups on the chromanol ring and not with the methyls on the phytyl side chain. Kagan et al. [29] proposed that a role of α-tocopherol is to bind any potentially dangerous free fatty acids that may appear in aging or diseased membranes. Thus, although general agreement exists that in some way α-tocopherol alters membrane properties, there remains considerable doubt as to exactly how. Here we use the techniques of fluorescence polarization, ultraviolet light absorbance and protection from fluorescence quenching by acrylamide, techniques which have previously been employed in α-tocopherol studies, to address these issues.

Materials and Methods

Materials. The following phosphatidylcholines were purchased from Avanti Polar Lipids (Pelham, AL): di-14:0 PC; di-16:0 PC; di-18:0 PC; di-18:1 PC; di-18:2 PC; di-α-18:3 PC; and 18:0, 18:1 PC. The mixed chain PCs (18:0,18:2 PC; 18:0,α-18:3 PC; and 18:0,22:6 PC) were synthesized from monostearoyl PC and the appropriate fatty acid anhydride (NuChek Prep, Elysian, MN). The procedure used was Keough's [30,31] and is outlined in a previous communication [32]. Potential oxidation of the unsaturated lipids was minimized by keeping the compounds out of light and under nitrogen at −40°C. Oxidation was detected by TLC [33], UV analysis [34] and gas chromatography [35]. Fatty acids were purchased from Sigma Chemical Co., St. Louis, MO and α-tocopherol from Serva Biochemicals (Westbury, NY). Molecular Probes (Eugene, OR) was the source of the fluorescence probes.

Lipid vesicles. Phospholipids, α-tocopherol, and where appropriate, fluorescent labels were codissolved in chloroform and then evaporated under nitrogen followed by vacuum pumping overnight. Lipids were hydrated in 20 mM phosphate, pH 7.0 buffer. The aqueous multilamellar vesicles (MLVs) containing the fluorescent labels were sonicated on ice for about 5 min using a Heat Systems W-380 Cell Disruptor. Titanium particles were removed from the resultant small unilamellar vesicles (SUVs) by a brief centrifugation.

Fluorescence. Fluorescence polarization was calculated from:

$$P = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + GI_{\perp}} \qquad (1)$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the horizontal and vertical components of emitted light and G is the grating factor (which corrects for instrument artifacts) [36]. Fluorescent intensities and P values were measured on a Perkin-Elmer MPF-66 Fluorescence Spectrophotometer interfaced to a Perkin-Elmer 7700 Professional computer and run by a PECLS program. Excitation was at 351 nm and emission at 430 nm for DPH, while the respective parameters for the AS probes were 341 nm and 446 nm. The probe/phospholipid ratio was 1/200 and the lipid concentration was 2.5 mM. Temperature was controlled to ±0.1 C° and the P values presented are the average of six determinations.

UV absorbance. UV absorbance measurements were made on a Perkin-Elmer Lambda 4C UV/Vis Spectrophotometer interfaced to a Perkin-Elmer 7700 Computer. The UV absorbance maximum of α-tocopherol is 213 nm. Addition of fatty acids or mixed chain PCs has the effect of lowering the absorbance at this maximum. By measuring the initial and final absorbances it is possible to calculate an interaction constant K for the α-tocopherol-fatty acid complex by:

$$K = \frac{A_0 - A}{A_0\,[\text{fatty acid}]} \qquad (2)$$

where A_0 and A are the absorbances at 213 nm in the absence and presence of fatty acid [14]. All vesicle measurements were made in the liquid crystalline state. For the organic solution experiments, ethanol was redistilled immediately before use.

Quenching by acrylamide. It has been previously shown that acrylamide can quench α-tocopherol fluorescence [21]. Here we employ this technique to monitor the extent of interaction between the vitamin and free fatty acids in redistilled, absolute ethanol and between the vitamin and the acyl chains of mixed chain phosphatidylcholines in lipid vesicles. α-Tocopherol was excited at 293 nm while emission was followed at 323 nm on a Perkin Elmer MPF-66 Fluorescence Spectrophotometer. Upon addition of acrylamide, fluorescence intensities decrease and the magnitude of the decrease is dependent on the amount of unsaturation present in the fatty acids. The initial (F_0) and final (F) α-tocopherol fluorescence intensities before and after

addition of acrylamide are determined and the extent of bleaching expressed as a Stern-Volmer plot [21].

Results

Steady-state fluorescence polarization of DPH and AS is sensitive to molecular motion within the bilayer interior. Order dominates for DPH, while for AS order and rate of motion contribute significantly to differing degrees dependent upon position of labeling [37]. The term fluidity will be employed in the latter case. The results presented in Fig. 1 for DPPC vesicles with either 0 or 10 mol% α-tocopherol show that with 12AS, α-tocopherol decreases fluidity (increases P) in the liquid crystalline state and increases fluidity (decreases P) in the gel state. This conclusion is complemented by measurements with the membrane interior probe DPH, which indicate increased and decreased order, respectively, above and below the phase transition. Polarization values for DPH in DPPC bilayers containing 0 and 10 mol% α-tocopherol at 30°C (gel state) and 50°C (liquid crystalline state) are included in Fig. 1. The phase transition displayed by DPPC is greatly broadened by the vitamin at 10 membrane

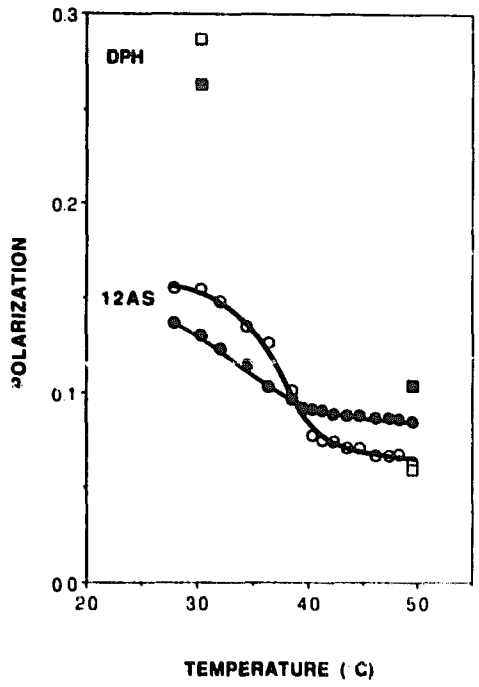

Fig. 1. Florescence polarization values for the membrane probe 12-AS in DPPC (di-16:0 PC) in the presence of 0 (○) and 10 (●) membrane mol% α-tocopherol at temperatures which span the DPPC phase transition (41.3°C). Points are also included for the membrane interior probe DPH at 0 (□) and 10 (■) membrane mol% α-tocopherol at temperatures below (30°C) and above (50°C) the phase transition of DPPC.

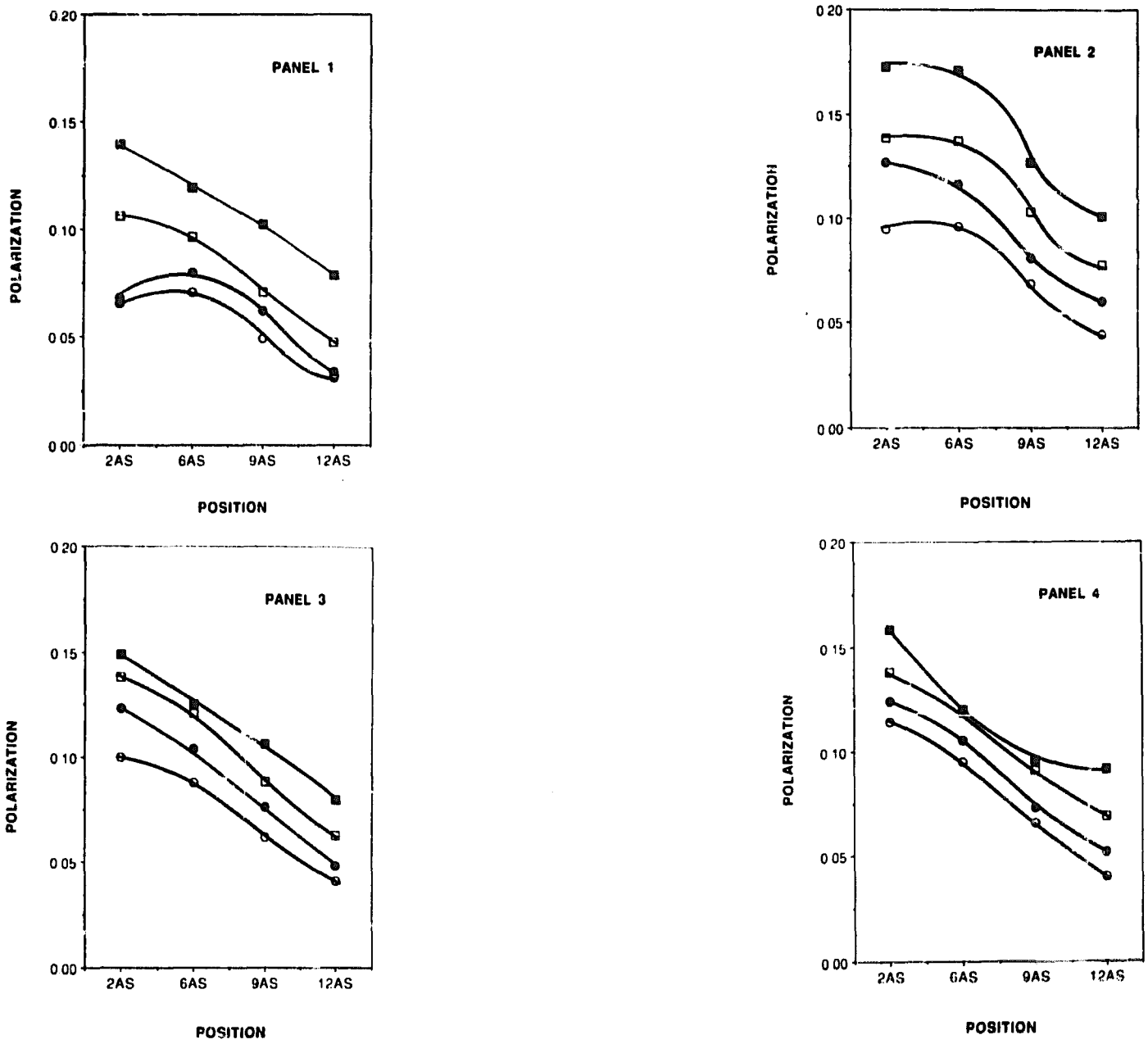

Fig. 2. Fluorescence polarization values for the membrane probes 2-AS, 6-AS, 9-AS and 12-AS in the presence of 0 (○), 5 (●), 10 (□) and 20 (■) membrane mol% α-tocopherol. Membranes are made of phosphatidylcholine in which both acyl chains are the same. (Panel 1) di-18:0 PC (65°C); (Panel 2) di-18:1 PC (35°C); (Panel 3) di-18:2 PC (35°C); (Panel 4) di-18:3 PC (35°C).

mol%. In agreement with DSC data [18], higher concentrations of α-tocopherol result in complete obliteration of the transition (results not shown). α-Tocopherol therefore affects DPPC bilayers in a similar fashion to cholesterol [38,39].

The effect as monitored by AS probes of α-tocopherol on phosphatidylcholine bilayers in which the acyl chains are symmetric (i.e. the *sn*-1 and *sn*-2 acyl chains are the same) is reported in Fig. 2. For all four lipids (di-18:0 PC, panel 1; di-18:1 PC, panel 2; di-18:2 PC, panel 3 and di-18:3 PC, panel 4) measurements were made in the liquid crystalline state where α-tocopherol decreases fluidity (increases P) relative to its membrane concentration. This effect is noted at positions near the aqueous interface (2-AS) through to the bilayer interior (12-AS). In agreement with the trend seen in our EPR data [40], there is a slightly smaller effect due to α-tocopherol noted with increasing unsaturations (e.g. for the 2-AS label the magnitude of the change in polarization between 0 and 20 mol% α-tocopherol, ΔP, decreases from di-18:1 PC (0.078) > di-18:2 PC (0.049) > di-18:3 PC (0.0439)). Similar small increases were also noted for ΔP in 6- and 9-AS positions. The explanation offered in the EPR work [40] proposed a relationship between α-tocopherol associated increases in order or microviscosity and the tightness of molecular packing within the phospholipid bilayer. The saturated lipid (DSPC, panel 1) was also tested with membranes in the liquid crystalline state but at a different temperature (65°C) and therefore is not quantitatively compared with the other lipids in this sequence.

Biological phosphatidylcholines do not normally contain identical *sn*-1 and *sn*-2 chains. Instead these lipids usually have a saturated fatty acid esterified to the *sn*-1 position with an unsaturated fatty acid at the *sn*-2 position. For this reason the effect of α-tocopherol on mixed acyl chain 18:0,18:1 PC, 18:0,18:2 PC and 18:0,18:3 PC was also investigated with AS probes (Fig. 3). The results are quite different than with the

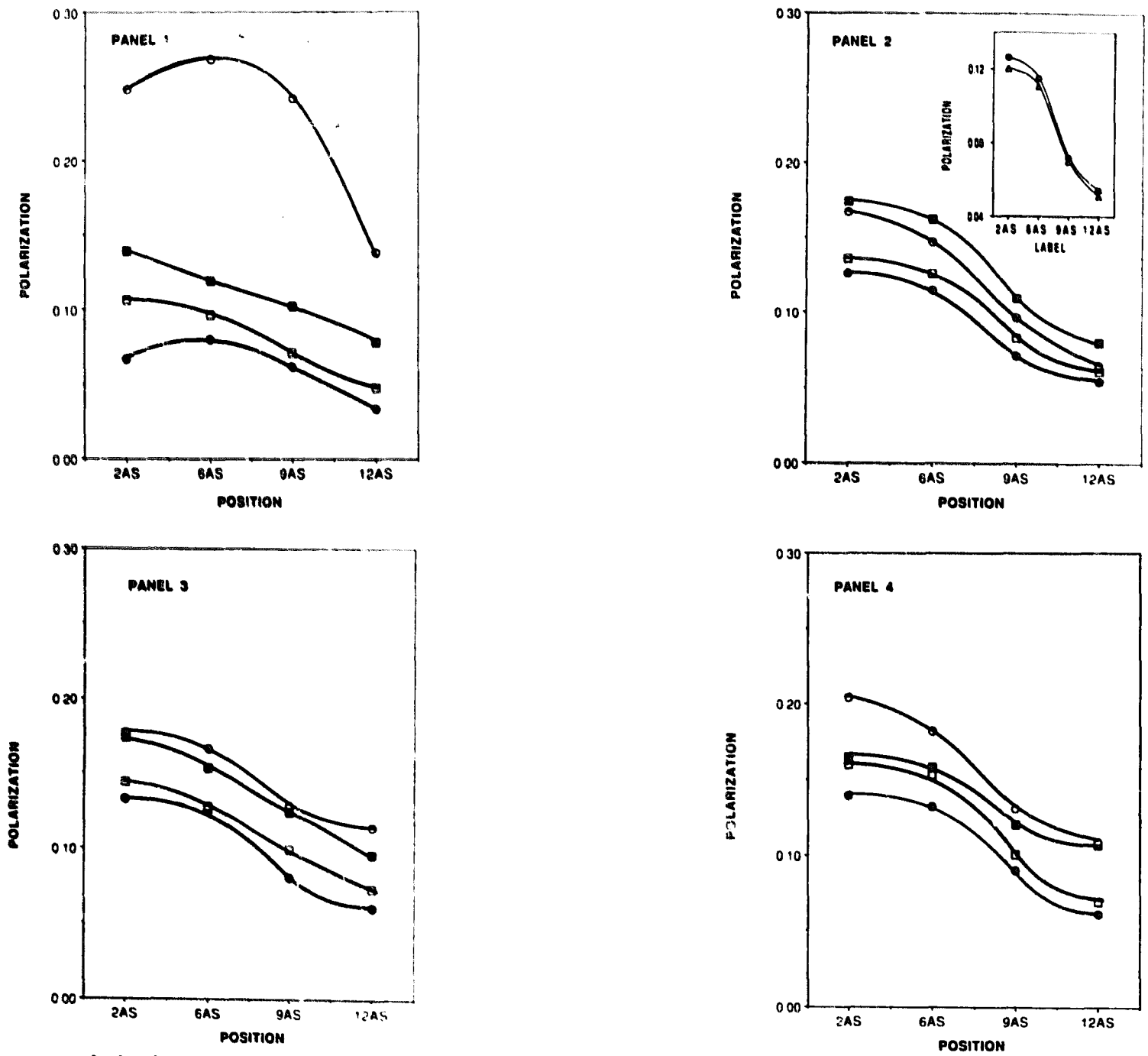

Fig. 3. Fluorescence polarization values for the membrane probes 2-AS, 6-AS, 9-AS and 12-AS in the presence of 0 (○), 5 (●), 10 (□) and 20 (■) membrane mol% α-tocopherol. Membranes are made of phosphatidylcholine in which the *sn*-1 position is stearic acid (18:0) and the *sn*-2 position contains various fatty acids. (Panel 1) di-18:0 PC (35°C); (Panel 2) 18:0,18:1 PC (35°C); (Panel 3) 18:0,18:2 PC (35°C); (Panel 4) 18:0,18:3 PC (35°C). The inset in panel 2 compares the effect of 2 (△) and 5 (●) mol% α-tocopherol.

symmetric diacyl lipids reported in Fig. 2. The inset to Fig. 3 demonstrates while 2 mol% α-tocopherol reduces P, higher concentrations increase P. Similar results were noted at positions 2, 6, 9 and 12 down the chain for each lipid tested and with additional mixed chain PCs not reported. Panel 1 reports the diacyl 18:0,18:0 PC in the gel state (35°C). By comparison, in the liquid crystalline state this lipid displayed a monotonic decrease in fluidity with increasing α-tocopherol.

Existence of α-tocopherol-fatty acid complexes is indicated on the basis of fatty acid induced decreases in absorbance at the short wavelength maximum (213 nm) of α-tocopherol [14,15]. The interaction constants for the various fatty acid-α-tocopherol complexes can be calculated via Eqn. 2 from the decrease in absorbance as a function of fatty acid concentration at constant concentration of α-tocopherol [14]. UV absorbance was used to demonstrate complexes between α-tocopherol (0.1 mM) and 0 to 4 mM of either free fatty acids or mixed acyl chain phosphatidylcholines (di-18:0 PC, 18:0,18:1 PC, 18:0,18:2 PC, 18:0,18:3 PC and 18:0,22:6 PC) in ethanol (results not shown). The magnitude of the interaction constant increases with the number of unsaturations for both the free fatty acids as well as for the mixed chain PCs in Fig. 4, panel 1, where a plot of log K vs. number of double bonds implies that perhaps similar complexes are formed between α-tocopherol and fatty acids whether the fatty acids are either free or esterified to a mixed chain phosphatidylcholine.

While previous publications presented UV absorbance evidence in favor of free fatty acid complexation with α-tocopherol in ethanol [14], it remained to be demonstrated that complexes similarly form between α-tocopherol and unsaturated fatty acids acylated to phosphatidylcholines in lipid bilayers. Therefore UV absorbance studies were made on liquid crystalline state (35°C) DMPC (5 mM) vesicles containing 0.5 membrane mol% α-tocopherol and mixed chain PCs at 0 to 5 membrane mol%. Greater decrease in UV absorbance for higher degrees of unsaturation was noted. The corresponding interaction constants that were calculated at 5 membrane mol% mixed chain PC's are reported in Fig. 4, panel 2. The fact that interaction constants can be calculated for α-tocopherol with very low concentrations of unsaturated fatty acids (in the form of mixed chain PCs) in the presence of large excess of saturated fatty acids (in the form of DMPC), indicate that α-tocopherol preferentially associates with fatty acids of increasing unsaturation in lipid bilayers.

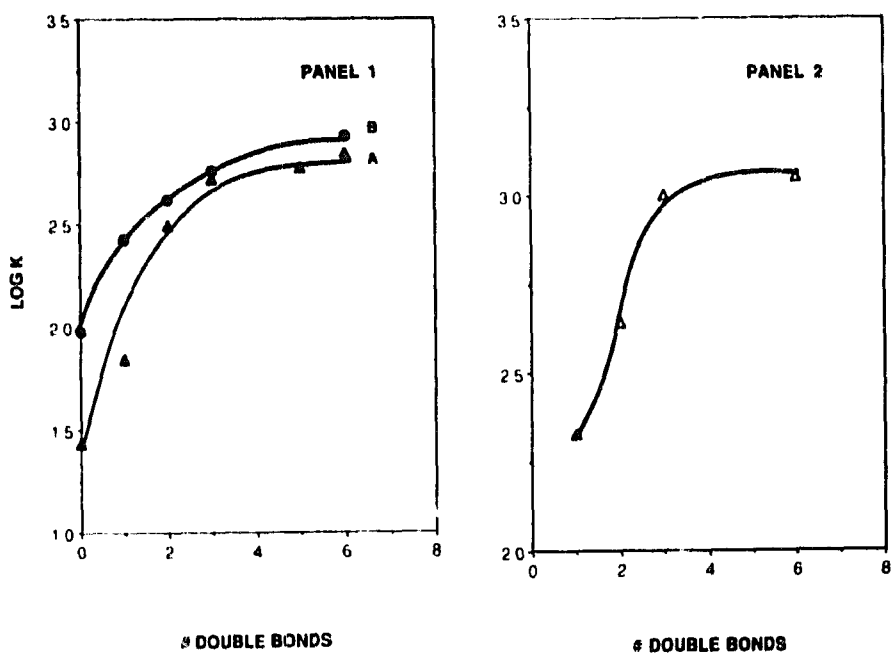

Fig. 4. (Panel 1) Semi-logarithmic plot of the association constants (25°C) of α-tocopherol (0.1 mM) to: (A) free fatty acids (0.25 mM); or (B) mixed acyl chain PCs in ethanol as a function of number of unsaturations in the fatty acids or PCs. (Panel 2) Semi-logarithmic plot of the association constants (35°C) of α-tocopherol (0.5 membrane mol%) to mixed chain PCs (5.0 membrane mol%) in DMPC lipid bilayers (5 mM vesicles in solution) as a function of the number of unsaturations in the *sn*-2 position of the PCs.

As an alternative approach, quenching of α-tocopherol fluorescence by acrylamide [21] was used to demonstrate complexation of the vitamin to unsaturated fatty acids in DMPC lipid bilayers as well as in organic solution. In Fig. 5, panel 1, α-tocopherol was incorporated at 5 membrane mol% into lipid vesicles (5 mM) composed of DMPC/mixed chain PC (4:1). The mixed chain PCs were: 18:0,18:1 PC; 18:0,18:2 PC; 18:0,α-18:3 PC; 18:0,22:6 PC or DMPC (control). Rapid fluorescence quenching was followed as acrylamide was added to the aqueous solution. Protection from quenching was related to the degree of acyl chain unsaturation. The least protection from acrylamide quenching was measured with the most saturated acyl chains. These results could indicate that either α-tocopherol is found at increasing depths in the bilayer [21] with increasing unsaturations (and hence is less exposed to the acrylamide) or else α-tocopherol may complex more strongly with the more unsaturated lipids.

The above possibilities can be distinguished by two experiments. Monitoring acrylamide quenching of α-tocopherol fluorescence in ethanol removes the orientation factor which results from α-tocopherol's incorporation into lipid bilayers. In Fig. 5, panel 2, it is evident that stearic acid (18:0) provides far less protection from acrylamide quenching in ethanol than does docosahexaenoic acid (22:6). Protection from quenching is related to the number of double bonds in the fatty acid. Acrylamide quenching rates were also measured for α-tocopherol (0.5 mol%) incorporated into bilayers composed of DPPC containing 10 mol% α-linolenic acid ($18:3\Delta^{9,12,15}$) or γ-linolenic acid ($18:3\Delta^{6,9,12}$). These membranes vary only in the position of the unsaturations in the linolenic acids. If quenching is related to the degree of exposure of

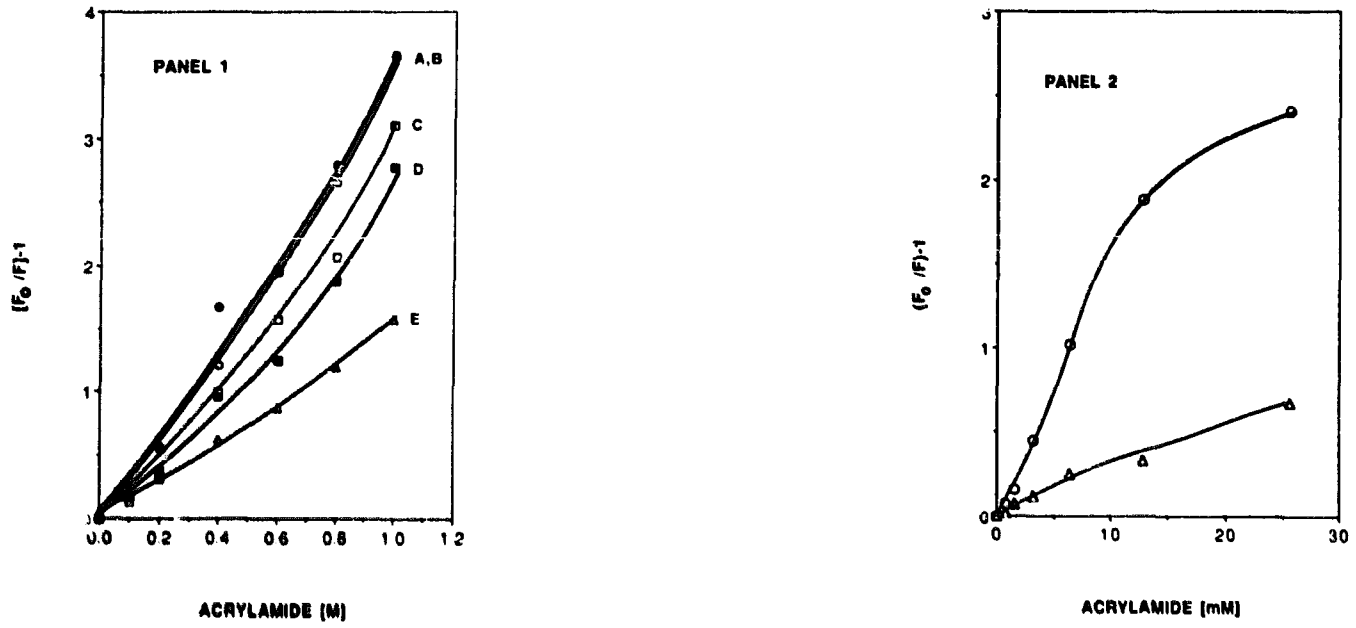

Fig. 5. (Panel 1) Protection of α-tocopherol (5 membrane mol%) from acrylamide fluorescence quenching by the acyl chains of phosphatidylcholines (5 mM) in lipid bilayers (35°C): (A) 100 mol% DMPC (○); (B) 80 mol% DMPC/20 mol% 18:0,18:1 PC (●); (C) 80 mol% DMPC/20 mol% 18:0,18:2 PC (□); (D) 80 mol% DMPC/20 mol% 18:0,18:3 PC (■); (E) 80 mol% DMPC/20 mol% 18:0,22:6 PC (△). (Panel 2) Protection of α-tocopherol (5 mol%) from acrylamide fluorescence quenching by free fatty acids (1.0 mM) in ethanol (25°C): (A) stearic acid (18:0), and (B) docosahexaenoic acid (22:6) in ethanol. Values are plotted as Stern-Volmer plots.

α-tocopherol to the aqueous interface and the carboxyl group of both fatty acids resides at about the same depth in the membrane, then the γ-linolenic acid membrane should be quenched to a greater extent than the α-linolenic acid membrane. Fig. 6 clearly shows that both membranes quench the same. Therefore we conclude that protection from acrylamide quenching is afforded by the number of acyl chain double bonds and not the position of the double bonds in the membrane. These results substantiate the UV studies and indicate that complexes between α-tocopherol and polyunsaturated fatty acids may exist in membranes as well as in organic solutions.

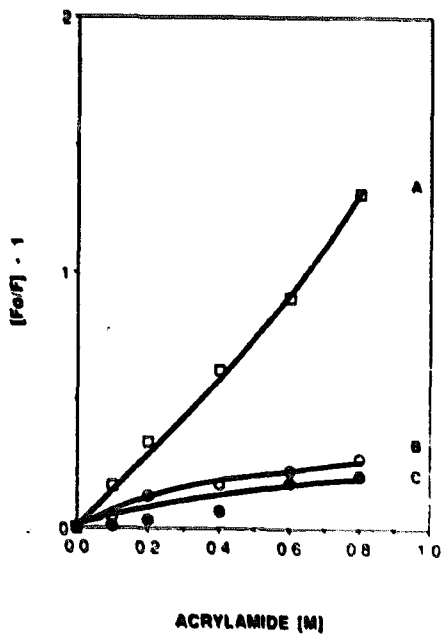

Fig. 6. Protection of α-tocopherol (5 mol%) from acrylamide fluorescence quenching by α-linolenic acid ($18:3\Delta^{9,12,15}$) and γ-linolenic acid ($18:3\Delta^{6,9,12}$) (10 mol%) in DPPC vesicles (5 mM) (47°C). Values are plotted as Stern-Volmer plots. (A) 100 mol% DPPC (□); (B) 90 mol% DPPC, 10 mol% α-linolenic acid (○); (C) 90 mol% DPPC, 10 mol% γ-linolenic acid (●).

Discussion

α-Tocopherol is a well studied membrane antioxidant [41,42]. Its function, in part, is to prevent oxidation of the most susceptible molecules found in nature, the long chain polyunsaturated fatty acids (PUFAs). By ultraviolet light and fluorescence measurements [14,15] complexes between free unsaturated fatty acids and α-tocopherol have been demonstrated in organic solution. Here we apply the same techniques to extend the list of PUFAs to the fish oil ω-3 fatty acid, docosahexaenoic acid (22:6) and demonstrate that similar complexes form whether the fatty acids are free or esterified to the *sn*-2 position of phosphatidylcholines (Figs. 4 and 5). Importantly our data suggest that complexes form in phospholipid bilayers as well as in ethanolic solution. The uv experiments show that α-tocopherol absorbance is affected by small amounts of unsaturated fatty acids in the presence of a large excess of saturated chains (unsaturated/saturated, 1:20) (Fig. 4, panel 2) indicating α-tocopherol's association is stronger with the more unsaturated fatty acids.

These results on artificial systems complement vitamin E experiments performed on various biological membranes. Some in vivo experiments indicate that α-tocopherol accumulates in membranes containing large amounts of unsaturated fatty acids [43]. If α-tocopherol does provide protection from lipid oxidation, it is in the membranes rich in PUFAs that the vitamin's concentration should be elevated. But even here, the amount of α-tocopherol present is still low. Ratios of α-tocopherol/PUFA have been reported between 1:2100 and 1:200 [43]. For the inner mitochondrial membrane a ratio of 1:4500, α-tocopherol/docosahexaenoic acid was found [43]. It is clear

that small amounts of α-tocopherol must protect a large number of PUFAs from oxidation.

The striking difference noted between the effect of α-tocopherol on fluidity of the diacyl PCs (Fig. 2) compared to that of the mixed chain PCs (Fig. 3), and the opposite effect of α-tocopherol on liquid crystal vs. gel state lipids (Fig. 1) may explain some of the discrepancies reported for the vitamin on various membrane properties. The effects of α-tocopherol on membranes are complicated and very phospholipid dependent. In addition most of the published α-tocopherol-phospholipid bilayer studies have used unrealistically high levels of the vitamin, up to 40 mol%. The mixed chain PC experiments reported here imply that one trend is measured with low levels (less than 2 mol%) α-tocopherol while a reverse trend is noted with higher levels. Using differential scanning calorimetry Micol et al. [44] have proposed that by 5 mol%, α-tocopherol induces lateral phase separation in phosphatidylethanolamine bilayers. Severcan and Cannistraro [7] have similarly concluded by EPR that α-tocopherol can induce two different phases in PC bilayers. Van Ginkel (personal communication) has noted that the orientation of the chromanol group varies with the concentration of the vitamin in the bilayer. Bellemare and Fragata [45] have reported that in unilamellar PC bilayers α-tocopherol reaches saturation levels at 5 mol%.

Phase separation in the presence of α-tocopherol is not universally concluded, however. A ^{2}H NMR study of 25 mol% selectively deuterated α-tocopherol incorporated into egg PC bilayers saw evidence of only one environment [45], although two component spectra (one for each phase) would be seen only if exchange between the phases is sufficiently slow. Studies of the effects of α-tocopherol on acyl chain order in saturated PC membranes and in membranes containing mixed chain PCs are similarly consistent with a single phase [12,46,47]. Also in favor of a single phase only is the single component character of EPR spectra recorded for PC membranes containing 0–20 mol% α-tocopherol [40]. In contrast to the current work, moreover, the EPR studies of mixed chain PCs detect monotonic increases in order and correlation time throughout the α-tocopherol concentration range employed. While differences between the spectroscopic techniques and the use of sonicated unilamellar vesicles (fluorescence studies) vs. multilamellar liposomes (EPR studies) are possible sources of the apparent discrepancies, the experiments indicate that α-tocopherol-membrane interactions are indeed complicated and data interpretation must be made with great care.

Our previous experiments have shown that despite not altering the fluidity of already unsaturated phospholipid bilayers, the presence of DHA makes membranes more permeable and susceptable to fusion [32]. A possible explanation is that the ω-3 fatty acid is not homogeneously distributed throughout lipid bilayers. Instead it may exist in high local concentrations in lipid domains where it does not alter the global fluidity but does severely affect permeability and fusion. If α-tocopherol complexes to DHA, as the experiments reported here suggest, then α-tocopherol would also be nonhomogeneously distributed in membranes. This is in agreement with the suggestion of McMurchie and McIntosh [17] who hypothesized α-tocopherol is inhomogeneously distributed in membranes and Ortiz et al. [18] who showed α-tocopherol partitions into more fluid domains. At present we are attempting to address this problem using ^{2}H-NMR compare the effect of α-tocopherol on spectra obtained from 16:0,16:0 PC/16:0,22:6 PC bilayers in which the individual PC components are deuterated [47].

The complexation of PUFAs to α-tocopherol in membranes may provide three distinct functions: an antioxidant, a structural component of membranes, and as a way to bind any potentially dangerous free fatty acids that may form in membranes.

Acknowledgement

This work has been supported by grants from the American Heart Association, Indiana Affiliate.

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