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The distribution of α-tocopherol in mixed aqueous dispersions of phosphatidylcholine and phosphatidylethanolamine

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Abstract

The effect of α-tocopherol on the structure and phase behaviour of mixed aqueous dispersions of phosphatidylcholine and phosphatidylethanolamine has been examined by synchrotron X-ray diffraction. Equimolar mixtures of dioleoylphosphatidylethanolamine:dioleoylphosphatidylcholine and dimyristoylphosphatidylcholine:dioleoylphosphatidylethanolamine did not show evidence of phase separation of an inverted hexagonal structure typical of α-tocopherol and phosphatidylethanolamine from lamellar phase. Mixed dispersions of dioleoyl derivatives of phosphatidylethanolamine: phosphatidylcholine (3:1) form a typical miscible gel phase at low temperatures but which phase separates into lamellar liquid–crystal and inverted hexagonal phases at temperatures greater than 65°C. The presence of 1, 2 or 5 mol% α-tocopherol caused a decrease in the temperature at which the inverted hexagonal phase appears. Phase separation of non-lamellar phase from lamellar gel phase can be detected in the presence of 7.5 and 10 mol% α-tocopherol, indicating a limited capacity of the phosphatidylcholine to incorporate α-tocopherol into the lamellar domain. A partial phase diagram of the ternary mixture has been constructed from the X-ray scattering data. It was concluded that there is no preferential interaction of α-tocopherol with phosphatidylethanolamine in mixed aqueous dispersions containing phosphatidylcholines.

Keywords: Phospholipid; Vitamin E; Model membrane; Antioxidant; X-ray diffraction

1. Introduction

α-Tocopherol is biologically and chemically the most active form of vitamin E in mammals. It is believed to have two primary functions in cells. Its major role is generally regarded to be its action as an antioxidant to prevent free radical damage to cells and tissues [1–6]. A subsidiary function is to stabilise the structure of cell membranes [7].

Free radicals are generated in biological systems during the catalytic cycle of some enzymes, during electron transfer through redox chains and exposure to a wide variety of external factors including metal ions, xenobiotic compounds, light and other forms of ionising radiation [3]. The reaction of free radicals with membrane constituents, especially the unsaturated membrane lipids, is believed to underlie a variety of disease states and the prevention of such dam-

Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; SAXS, small-angle X-ray scattering ($2\theta = 0.043°$–$7.9°$); WAXS, wide-angle X-ray scattering ($2\theta = 8°$–$60°$); L_α, lamellar liquid–crystalline phase; L_β, lamellar gel phase; H_{II}, inverted hexagonal phase

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age, and is said to be a primary function of vitamin E. Vitamin E is known to react with many different types of oxidants but its principal antioxidant action is as a peroxyl radical scavenger [2]. Evidence for the role of vitamin E as a membrane stabiliser has been obtained from paramagnetic resonance studies of the effect of free fatty acids and lysophospholipids on the stability of phospholipid model membranes [7–9]. These showed that vitamin E tends to from complexes with these membrane destabilising agents thereby counteracting their effects and rendering the membrane more stable.

Biological membranes contain a complex assortment of polar lipids differing in polar head group and hydrocarbon substituents. The distribution of lipids within the bilayer matrix is not homogeneous and there is convincing evidence of both lateral and transverse asymmetry of lipid components [10–12]. The amount of α-tocopherol in membranes generally falls within the range 0.1–1 mol% of phospholipids in most membranes [13] and can thus be regarded as a relatively minor lipid constituent. Nevertheless, the creation of enriched domains of α-tocopherol may result in localised effects on membrane structure and stability. Model membrane studies using single molecular species of phospholipids have provided evidence for the existence of stoichiometric complexes between specific phospholipids and α-tocopherol [14–17]. There have, however, been few studies to determine whether α-tocopherol preferentially interacts with specific phospholipids or partitions into particular phospholipid domains. From a calorimetric study of the effect of α-tocopherol on aqueous dispersions of phospholipid mixtures, Ortiz et al. [18] concluded that α-tocopherol tends to partition into the most fluid phases of the bilayer irrespective of whether this was phosphatidylcholine (PC) or phosphatidylethanolamine (PE). This conclusion appeared to be inconsistent with the formation of complexes with saturated PCs and induction of non-bilayer phases in PEs deduced from studies of aqueous dispersions of binary mixtures of α-tocopherol and phospholipids [14–17].

The present synchrotron X-ray diffraction study was aimed to determine whether complex formation could be detected in aqueous dispersions of mixed phospholipids in the presence of α-tocopherol. Synchrotron X-ray diffraction methods have been used because the method not only allows direct structural assignment of the phospholipid phase but also provides information on acyl chain order, changes of which are known to be highly correlated with enthalpy measurements obtained from calorimetry [19]. The study focussed on unsaturated molecular species of phospholipid as these are more relevant models of the membrane lipid bilayer matrix.

2. Materials and methods

DMPC (dimyristoylphosphatidylcholine), DOPE (dioleoylphosphatidylethanolamine) and DOPC (dioleoylphosphatidylcholine) were purchased from Avanti Polar Lipids (Alabaster) and α-tocopherol from Acros Organics (Geel, Belgium). The lipids were dissolved in chloroform and mixed in appropriate proportions to achieve the desired molar fractions. The solvent was evaporated under a stream of oxygen-free dry nitrogen and stored for 24 h under vacuum to remove any remaining traces of solvent. The lipid mixtures were hydrated at temperatures where the lipids were in the fluid phase (>80°C) for at least 1 h and dispersed using a rotamixer until homogeneous dispersions were obtained. The lipid dispersions were stored at 0–4°C for 24 h before examination. The method of preparation and storage gave reproducible phase behaviour when samples prepared at different times were examined by X-ray diffraction. There was no evidence of any radiation damage to the samples with the experimental protocols employed.

Synchrotron X-ray diffraction experiments were performed using a monochromatic (0.15405 nm) focused X-ray beam at station 8.2 of the Daresbury Synchrotron Radiation Laboratory, UK. The camera configuration allowed detection of small-angle and wide-angle X-ray scattering (SAXS and WAXS) with a minimum of parallax error [20]. The beamline generates a flux of 4×10^{10} photons per second with a focal spot size of 0.3×3 mm^2 (V×H) when the synchrotron radiation source is operating at a nominal 200 mA. The samples were mounted in a slot (1×5 mm) cut in a 1 mm thick copper plate sandwiched between a pair of thin mica sheets. The sandwich was clamped to the silver block containing the temperature sensing and modulating

elements of a cryomicroscope stage (Linkam Scientific Instruments Ltd., UK). X-ray scattering intensities at small angles (SAXS, $2\theta = 0.043°–7.9°$) were recorded using a multiwire quadrant detector. X-ray scattering intensities at wide angles (WAXS, $2\theta = 8°–60°$) were recorded using an INEL curved linear-wire detector (INstrumentation Electronique, France). Data were acquired in 400 consecutive time frames of 5 s and separated by a dead time between frames of 50 ms. Experimental data were analysed using the OTOKO software (EMBL, Hamburg, Germany) programme [21]. Scattering intensities at low angles were corrected for fluctuations in beam intensity and detector response recorded from an ^{55}Fe source. Spatial calibrations were obtained using 21 orders of wet rat tail collagen ($d = 67$ nm) [22]. The scattering intensity data recorded by the INEL detector were corrected for scattering from an empty cell and spatial calibrations were established from high-density polyethylene (0.4166, 0.3780, 0.3014 nm) [23]. Samples were heated to about 80°C prior to recording the first cooling scan to avoid unnecessary exposure of the sample to the X-ray beam. The reciprocal spacing $S = 1/d = 2\sin(\theta)/\lambda$, where d, λ, θ are the repeat distance, X-ray wavelength and the diffraction angle/2, respectively.

3. Results

To examine the effect of α-tocopherol on the structure of unsaturated phospholipids, the thermotropic phase behaviour of DOPC and DOPE dispersions containing α-tocopherol was examined by synchrotron X-ray diffraction methods. These results were then compared with the effect of α-tocopherol on mixed aqueous dispersions of the two phospholipids to investigate whether any preferential interactions with either phospholipid class could be identified.

3.1. Interaction of α-tocopherol with DOPC

Firstly, dispersions of DOPC containing up to 20 mol% α-tocopherol were examined to investigate the effect of α-tocopherol on the lamellar structure of the fluid phase of DOPC. The results showed that the presence of α-tocopherol does not cause any change in the lamellar structure of this phospholipid. This can be seen in Fig. 1, which shows static SAXS patterns recorded from aqueous dispersions of DOPC containing up to 20 mol% α-tocopherol at −15 and 10°C, respectively. Three orders of a bilayer repeat of 5.6 nm can be observed in mixtures containing 0, 1, 10 and 20 mol% α-tocopherol at −15°C (Fig. 1a). The acyl chain packing observed from the WAXS intensity profiles (data not shown) indicates the hydrocarbon region was disordered at −15°C. Dispersions examined at 10°C (Fig. 1b) showed that the bilayer repeat increases to 6.5 nm; this is due to hydration of the multilamellar dispersion when extra lamellar ice melts at 0°C. Another feature seen at 10°C is the increase in intensity of the second-order lamellar repeat reflection and a virtual disappearance of the third-order reflection. The results suggest that α-tocopherol mixes ideally with DOPC bilayers in the fluid phase at temperatures both above and below ice melting. Similar results have been reported in dispersions of mixtures of α-tocopherol with saturated diacyl PCs [26], in which only lamellar structures were observed in mixtures of α-tocopherol with dilauroyl-, dimyristoyl- and distearoyl-molecular species of the phospholipid. It infers that α-tocopherol

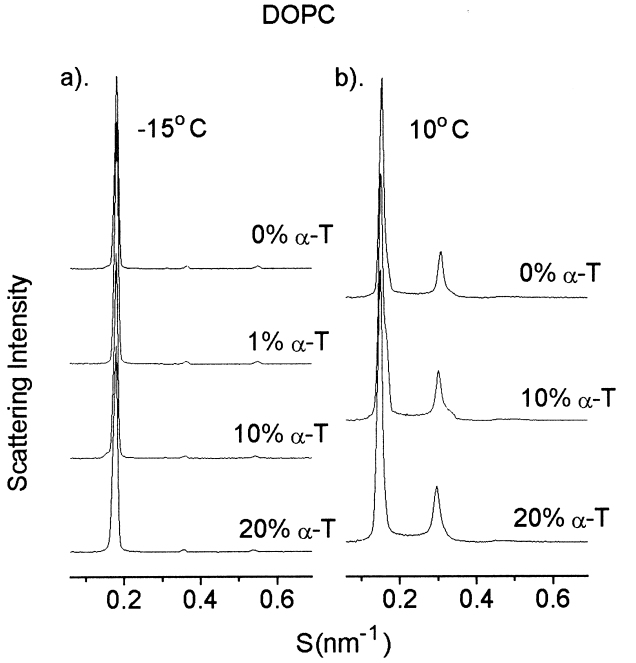

Fig. 1. Static SAXS intensity profiles as a function of reciprocal spacing of fully hydrated dispersions of DOPC containing indicated amounts of α-tocopherol recorded at (a) −15°C and (b) 10°C, respectively.

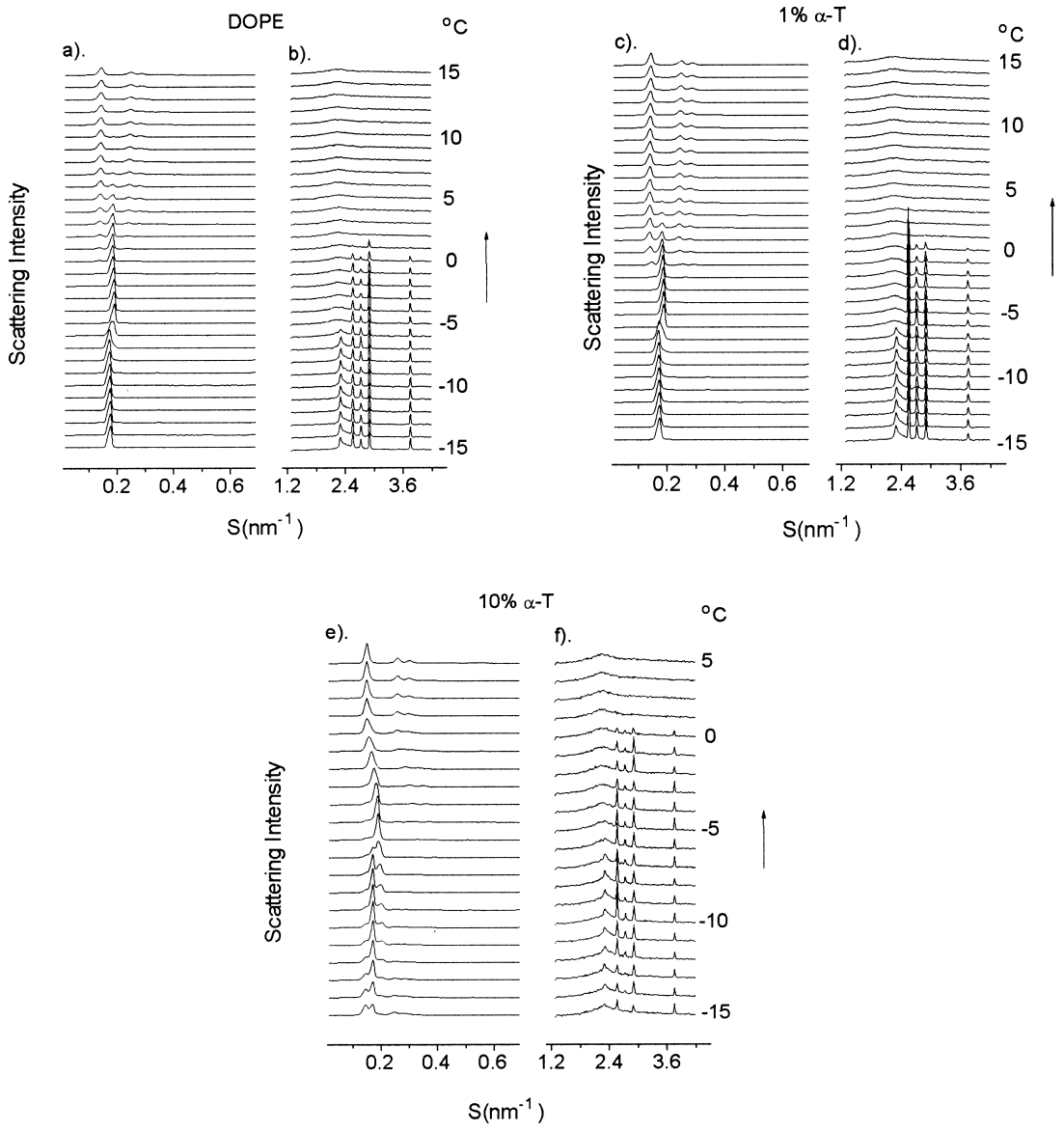

Fig. 2. Plots of successive SAXS (a,c,e) and WAXS (b,d,f) intensity profiles versus reciprocal spacing recorded during heating of a fully hydrated dispersions of DOPE containing (a,b) 0, (c,d) 1 and (e,f) 10 mol% α-tocopherol from −15°C at 1°/min. Each diffraction pattern represents scattering accumulated in 5 s.

distributes randomly in PC bilayers or that any α-tocopherol-enriched domains have an identical bilayer repeat to that of the corresponding pure phospholipid.

3.2. Interaction of α-tocopherol with DOPE

Plots of X-ray diffraction patterns recorded during a heating scan of a dispersion of DOPE in the absence of α-tocopherol are shown in Fig. 2a,b. It can be seen that the phospholipid undergoes a lamellar gel phase (L_β) → lamellar liquid–crystalline phase (L_α) transition at −6.5°C and a L_α to inverted hexagonal phase (H_{II}) transition at about 6°C. The gel phase transition is characterised by a decrease of the lamellar repeat spacing from 5.8 nm (−10°C) to 5.3 nm (3°C) seen in the SAXS region and a change from a sharp diffraction peak at 0.43 nm (−10°C) to a broadened peak centred at 0.45 nm (3°C) in the WAXS region signifying a disordering of the acyl

chains. The sharp peaks in the WAXS region centred at spacings corresponding to 0.39, 0.37, 0.34 and 0.27 nm represent reflections from hexagonal ice and disappear at 0°C. The H_{II} phase appears in the SAXS profile at about −1°C and replaces the L_α phase completely when the temperature reaches about 8°C.

The effect of α-tocopherol on the phase behaviour of DOPE is illustrated in Fig. 2c–f, which shows SAXS and WAXS intensity patterns recorded during heating scans of aqueous dispersions of DOPE containing 1 and 10 mol% α-tocopherol, respectively. The presence of α-tocopherol does not result in a significant shift in temperature of the $L_\beta \rightarrow L_\alpha$ transition but the appearance of H_{II} in the diffraction patterns occurs at progressively lower temperatures. Thus, the presence of 1 mol% α-tocopherol causes the $L_\alpha \rightarrow H_{II}$ transition to shift to about 1°C, with 10 mol% α-tocopherol H_{II} can be detected in the SAXS intensity profile at −15°C, which is well below the main transition temperature (−6.5°C) of pure DOPE. A similar effect of α-tocopherol has previously been observed in mixtures of α-tocopherol and saturated molecular species of PEs [14–16].

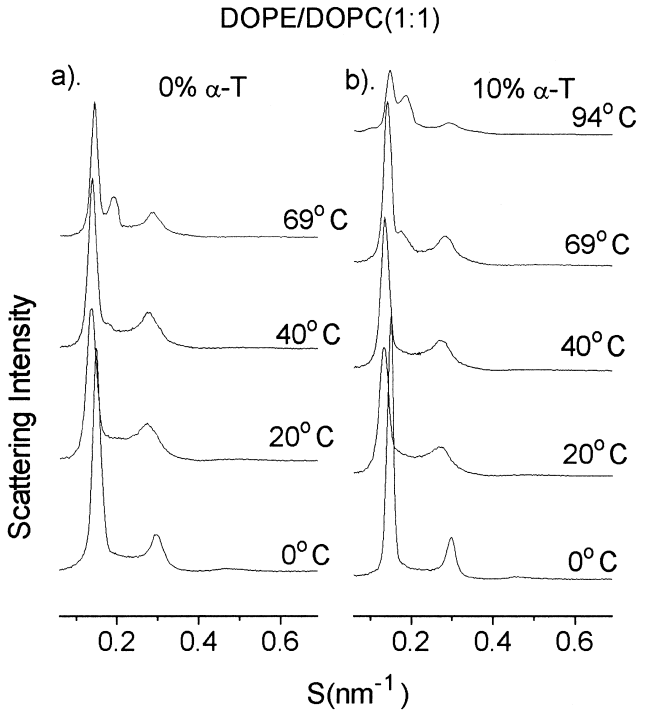

Fig. 3. Static SAXS intensity profiles as a function of reciprocal spacing of fully hydrated dispersions of DOPE/DOPC (1:1) containing (a) 0 and (b) 10 mol% α-tocopherol recorded at the temperatures indicated.

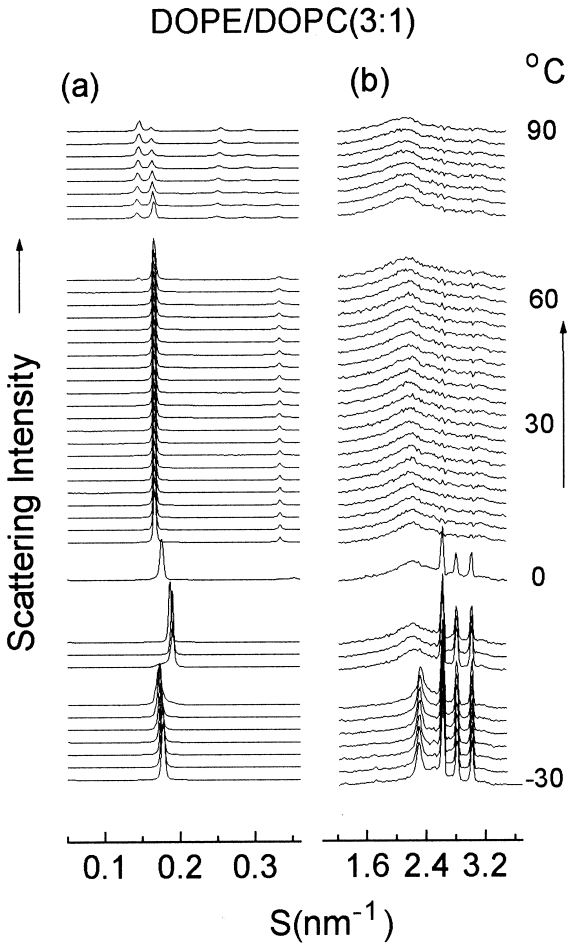

Fig. 4. Plots of successive SAXS (a) and WAXS (b) intensity profiles versus reciprocal spacing recorded from a fully hydrated dispersion of DOPE/DOPC (3:1) during a heating scan from −30°C to 90°C at 5°/min. Each diffraction pattern represents scattering accumulated in 20 s.

3.3. *Effect of α-tocopherol on mixtures of DOPE/DOPC*

Mixed aqueous dispersions of equimolar amounts of DOPE and DOPC containing 0 and 10 mol% α-tocopherol were first examined by synchrotron X-ray diffraction methods. Fig. 3a,b shows plots of static SAXS intensity patterns of aqueous dispersions of DOPE/DOPC (1:1) containing 0 and 10 mol% α-tocopherol, respectively, recorded at different temperatures between 0 and 94°C. It can be seen that at temperatures up to 40°C only lamellar phase was observed. The lamellar phase has a repeat spacing of 6.6 nm at temperatures below freezing and this in-

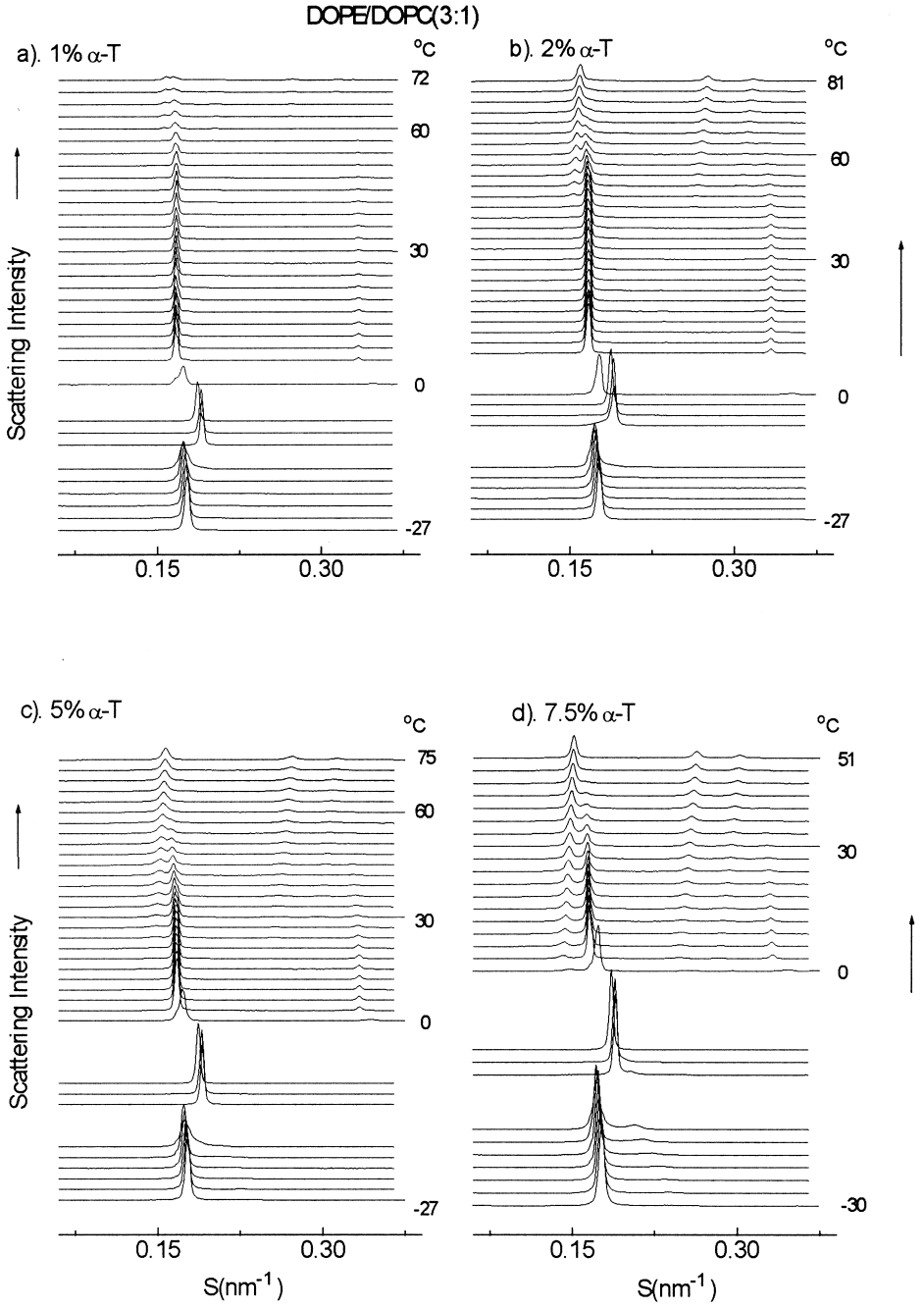

Fig. 5. Plots of successive SAXS intensity profiles versus reciprocal spacing recorded from codispersions of DOPE/DOPC (3:1) containing (a) 1, (b) 2, (c) 5 and (d) 7.5 mol% α-tocopherol during heating scans from −30°C at 5°/min. Each diffraction pattern represents scattering accumulated in 20 s.

creases to about 7 nm when water released from ice melting enters the multibilayer structure. Phase separation was observed at 69°C in mixtures with and without 10 mol% α-tocopherol, and this is characterised by an additional peak at 5.3 nm in the SAXS profile. Heating mixtures containing 10 mol% α-tocopherol to 94°C did not indicate the presence of any non-lamellar phase in the dispersion. This finding tends to exclude the possibility of interaction of α-tocopherol with DOPE domains so as to induce H_{II} seen with mixtures of the pure phospholipid. The results suggest that α-tocopherol preferentially inter-

acts with either DOPC or a mixed phase of DOPC/DOPE in bilayer configuration.

To determine the effect of α-tocopherol on mixtures in which the tendency of DOPE to form non-bilayer phases was not markedly suppressed by DOPC, studies of mixed aqueous dispersions of DOPE/DOPC (3:1) were undertaken. The phase behaviour of this mixture has been examined by several groups and it has been found to exhibit cocrystallisation in formation of a gel phase at low temperature [24,25]. Dispersions of the mixture of DOPE/DOPC (3:1) containing 0, 1, 2, 5, 7.5, 10 mol% α-tocopherol were examined by synchrotron X-ray diffraction methods to identify any effects on the phase behaviour of the mixture. Plots of X-ray diffraction patterns recorded during a heating scan of the phospholipid mixture in the absence of α-tocopherol (Fig. 4) show that the mixture undergoes a $L_\beta \rightarrow L_\alpha$ transition at −9.3°C. This coincides with a decrease of the lamellar repeat spacing from 5.6 nm (−30°C) to 5.2 nm (−9°C) in the SAXS region and a change from a symmetrical diffraction peak at 0.43 nm (−30°C) to a broadened peak centred at 0.46 nm (−9°C) in the WAXS region. The sharp peaks in the WAXS region centred at spacings corresponding to 0.39, 0.37 and 0.34 nm represent reflections from hexagonal ice and can be seen to disappear at 0°C. The lamellar d-spacing of liquid–crystalline phase increases significantly with increasing temperature until the ice melting point, whereupon it becomes stable at a d-spacing of 5.9 nm. The H_{II} phase first appears in the SAXS intensity profile at about 66°C and coexists with L_α phase such that at 90°C the intensity of the first-order lamellar repeat of the L_α phase is about 25% of that recorded at 30°C. This scattering intensity ratio represents the maximum phase separation of DOPE into H_{II} phase from DOPC in L_α phase that is achieved at 90°C.

The effect of α-tocopherol on the phase behaviour of the DOPE/DOPC (3:1) mixture is illustrated in Fig. 5. Fig. 5a shows SAXS patterns recorded during heating of an aqueous dispersion of the DOPE/DOPC (3:1) mixture containing 1 mol% α-tocopherol. The presence of α-tocopherol causes a shift in temperature of the $L_\beta \rightarrow L_\alpha$ transition to −10.3°C and the initial appearance of H_{II} phase at 54°C rather than at 66°C. The H_{II} phase coexists with L_α phase, which it progressively replaces with increasing temperature. SAXS intensity patterns recorded during heating of mixed aqueous dispersions containing 2, 5 and 7.5 mol% α-tocopherol in DOPE/DOPC (3:1) are shown in Fig. 5b–d, respectively. It can be seen that the $L_\alpha \rightarrow H_{II}$ phase transition temperature decreases with increasing proportions of α-tocopherol in the mixture. Furthermore, additional weak broad peaks (centred at 4.8 nm at −12°C) are observed at temperatures below the main transition that coexist with the gel phase peak in the mixture containing 7.5 mol% α-tocopherol (Fig. 5d).

The effect of higher proportions of α-tocopherol are presented in Fig. 6 which shows X-ray diffraction patterns recorded during a heating scan of a mixed aqueous dispersion DOPE/DOPC (3:1) containing 10 mol% α-tocopherol from −24°C to 81°C. At the initial temperature, an L_β phase, characterised by a sharp wide-angle reflection at 0.43 nm (which can be clearly distinguished from ice reflections at 0.39, 0.37 and 0.34 nm, respectively) coexisting with an additional peak (centred at 4.8 nm at −12°C) is observed.

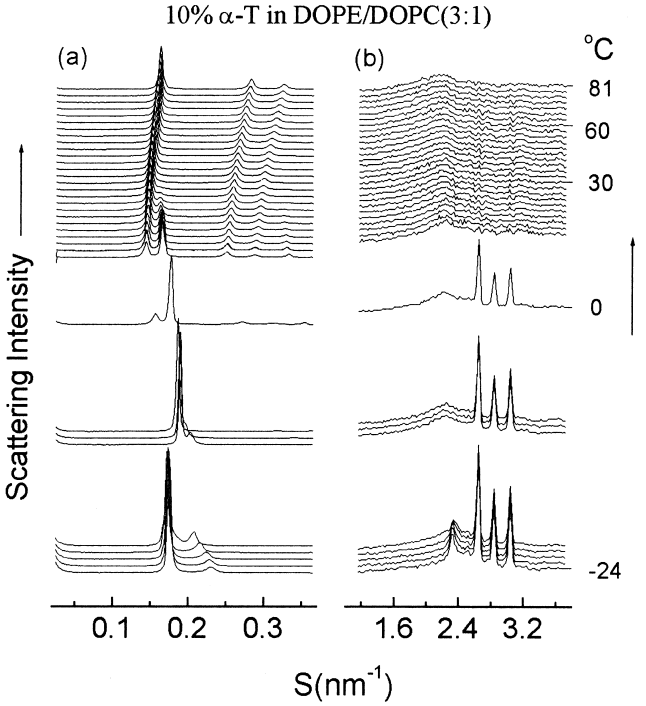

Fig. 6. Plots of successive SAXS (a) and WAXS (b) intensity profiles versus reciprocal spacing recorded from a codispersion of DOPE/DOPC (3:1) containing 10 mol% α-tocopherol during a heating scan from −24°C to 81°C at 5°/min. Each diffraction pattern represents scattering accumulated in 20 s.

With heating, an $L_\beta \rightarrow L_\alpha$ phase transition takes place at −11°C and this is progressively replaced by an H_{II} phase while remaining traces of the L_α phase disappear at 33°C. The d-spacing of the H_{II} phase decreases with increasing temperature.

The effect of α-tocopherol on the structure of mixed aqueous dispersions of DOPE/DOPC (3:1) is summarised in Fig. 7a, which shows static SAXS intensity profiles of mixtures containing different concentrations of α-tocopherol recorded at −15°, 15° and 45°C, respectively. This analysis allows confident assignment of the different phases induced by the presence of α-tocopherol. Thus at temperatures below the $L_\beta \rightarrow L_\alpha$ phase transition (−15°C), an additional peak at a d-spacing of about 5.1 nm first appears in mixtures containing 2 mol% α-tocopherol and its intensity increases with increasing α-tocopherol in the mixture. At temperatures above the gel to liquid–crystal phase transition, an H_{II} phase can be seen in mixtures containing 5 mol% or more α-tocopherol (15°C) and its intensity increases with increasing α-tocopherol in the mixture. Upon heating to 45°C, only H_{II} phase can be detected in the mixtures containing 7.5 mol% or more α-tocopherol. The effect of α-tocopherol on the phase behaviour of DOPE/DOPC (3:1) mixed dispersion can be seen from the relationship between d-spacing and temperature shown in Fig. 7b. This shows that α-tocopherol causes a progressive decrease in the temperature of the L_β to L_α phase transition and a marked decrease in the temperature of L_α to H_{II} phase transition. With increasing α-tocopherol in the mixture, the temperatures where H_{II} phase first appears and L_α phase disappears both decrease. Similar behaviour has been observed in mixtures of pure saturated PEs with α-tocopherol in which α-tocopherol-enriched domain formed H_{II} structure at temperatures below the main gel to liquid–crystal phase transition of the corresponding phospholipid [14–16]. The additional peaks observed in the gel phase can be seen with increasing α-tocopherol content to be a tocopherol-enriched domain forming an H_{II} phase. The repeat spacing increases dramatically reaching a maximum at the main gel to liquid–crystalline phase transition whereupon the spacing progressively decreases with increasing temperature.

The phase formed by DOPE/DOPC (3:1) dis-

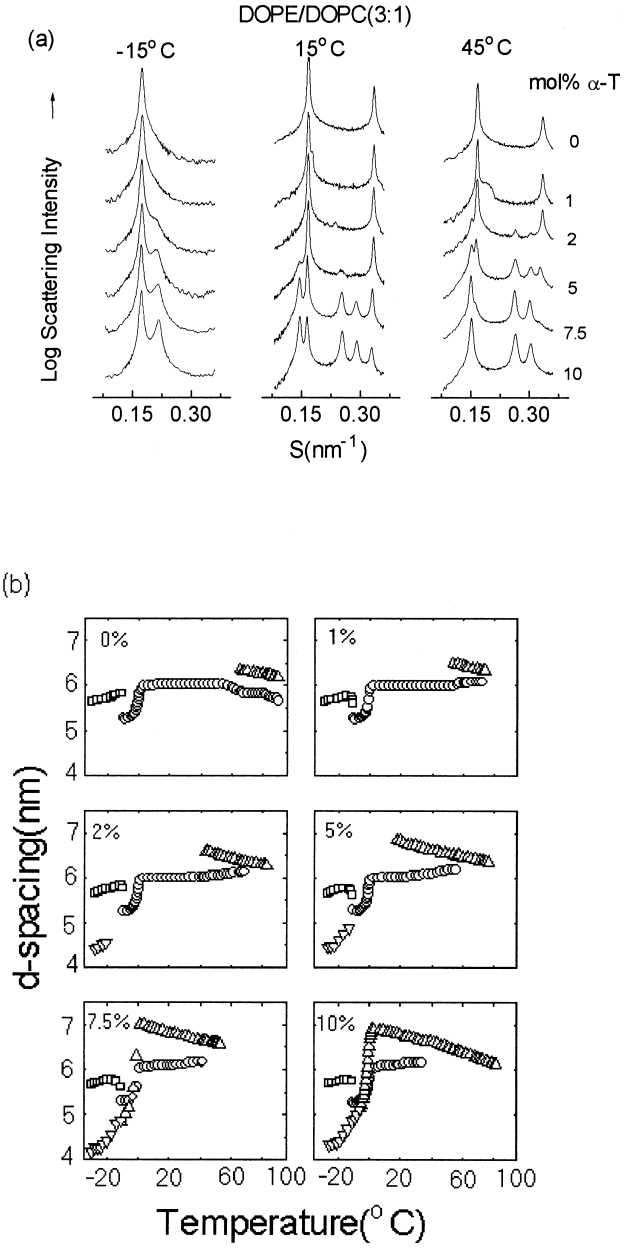

Fig. 7. (a) Static SAXS intensity profiles as a function of reciprocal spacing of fully hydrated dispersions of DOPE/DOPC (3:1) containing indicated amounts of α-tocopherol recorded at −15°C, 15°C and 45°C. Scattering intensities are plotted on a logarithmic scale to emphasise the minor bands. (b) Plots of the d-spacing of L_β (□), L_α (○) and H_{II} present with L_β (▽) or L_α (△) phases as a function of temperature of mixed aqueous dispersion of DOPE/DOPC (3:1) containing indicated amounts of α-tocopherol.

persed in excess water depends on the temperature and the amount of α-tocopherol incorporated into the mixture (see Figs. 5 and 6). In dispersions of pure phospholipid at temperatures above 90°C, a phase separation occurs between DOPE, which forms H_{II} structure, and DOPC, which exists in L_α phase. The ratio of X-ray scattering intensity from the first-order reflection from these structures (I_H/I_L) is constant at 1.7. The temperature at which the ratio of scattering intensities reaches a value of 1.7 is found to decrease with increasing amount of α-tocopherol in the mixture. This can be seen in Fig. 8a, which shows a plot of I_H/I_L as a function of the mol% α-tocopherol in the mixture. It may be inferred that α-tocopherol decreases the temperature whereupon phase separation takes place. The X-ray scattering data have been used to construct a partial phase diagram of DOPE/DOPC (3:1) plus α-tocopherol up to 10 mol% in excess water. This is presented in Fig. 8b. It can be seen that α-tocopherol firstly interacts with DOPC or the complex of DOPE/DOPC to form α-tocopherol-enriched domain L*, which has a lamellar structure. It is not clear whether this lamellar α-tocopherol-enriched domain has a defined stoichiometry of α-tocopherol and phospholipids, but at some point a limit to the amount of α-tocopherol that can be incorporated into this lamellar phase is reached and the excess α-tocopherol begins to interact with DOPE. The (DOPE+α-tocopherol) domain forms H_{II} structure, which can be detected even at temperatures well below the main phase transition of the mixture containing higher proportions of α-tocopherol. Temperature is another factor that influences the distribution of α-tocopherol within the mixed dispersion. With increasing temperature, the amount of H_{II} (DOPE+α-tocopherol) domain increases at the expense of the L* domain. When the temperature reaches the line where (I_H/I_L) is 1.7 (dashed line in Fig. 8a), the amount of the H_{II} (DOPE+α-tocopherol) domain is approximately 3-fold that of the L* domain as judged by the relative intensities of the SAXS peaks. One interpretation could be that this line represents a situation where all of the DOPE in the mixture forms an H_{II} (DOPE+α-tocopherol) domain, and the remaining L* domain consists entirely of (DOPC+α-tocopherol). Above this line, these two domains of L* and H_{II} (DOPE+α-tocopherol) gradually merged into single H_{II} (DOPC+DOPE+α-tocopherol) domain with increasing temperature.

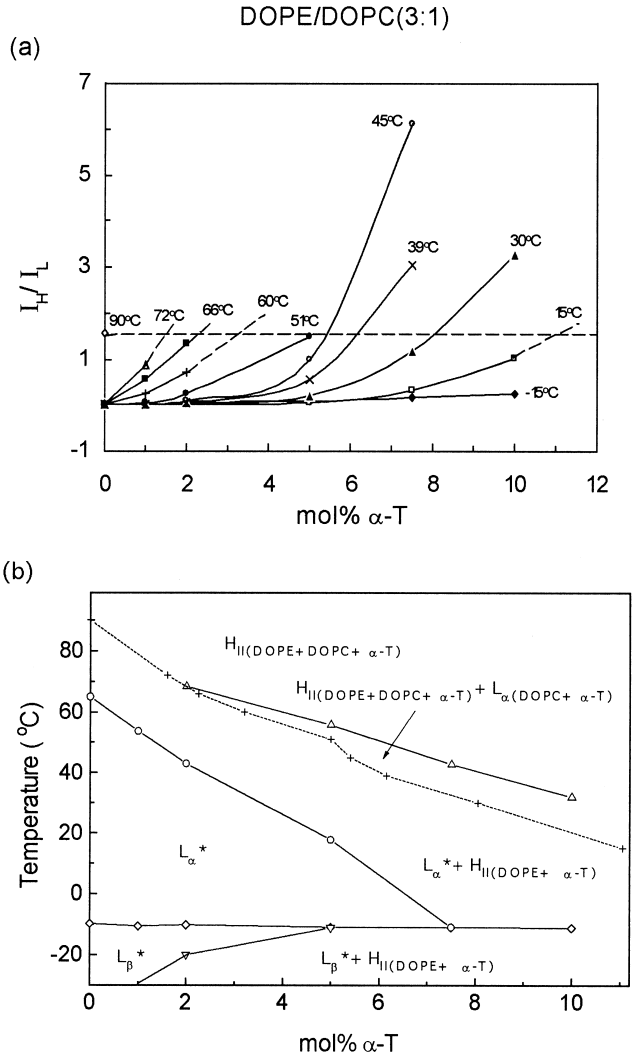

Fig. 8. (a) Comparison of the ratio of normalised X-ray scattering intensities of the first-order diffraction peaks of inverted hexagonal and lamellar phases, I_H/I_L, recorded at designated temperatures plotted as a function of α-tocopherol in the mixture of DOPE/DOPC (3:1). (b) Partial phase diagram of the mixture of DOPE/DOPC (3:1) and α-tocopherol. The dotted line in both figures represents values of $I_H/I_L = 1.7$. L* represents α-tocopherol-enriched domains of DOPC or DOPE/DOPC.

3.4. *Effect of α-tocopherol on mixtures of DOPE/DMPC (1:1)*

An additional series of experiments were performed to compare the effect of α-tocopherol on un-

saturated phospholipid mixtures with mixtures in which DMPC substituted for DOPC.

Mixed dispersions of equimolar proportions of DOPE and DMPC containing up to 10 mol% α-tocopherol were examined by synchrotron X-ray diffraction methods. The effect of 10 mol% α-tocopherol on the structure of the dispersion is illustrated in Fig. 9. This shows some static SAXS and WAXS intensity patterns recorded from aqueous dispersions of DOPE/DMPC mixtures containing 0 and 10 mol% α-tocopherol, respectively, at four temperatures spanning the $L_\beta \rightarrow L_\alpha$ phase transition temperature. It can be seen from the WAXS intensities that the lipid is in a gel phase at temperatures less than −5°C and the sharp SAXS peaks indicate that the lamellar phase (d-spacing 6.1 nm) is highly ordered. The bilayer repeat increases to 8.4 nm at 10°C and 20°C because water penetrates into the multilamellar structure as the ice melts. The peaks in both the SAXS and WAXS regions also become considerably broadened on transformation into the lamellar L_α phase, suggesting that the phase is somewhat disordered. Significantly, the presence of 10 mol% α-tocopherol does not induce formation of H_{II} even at temperatures above the main transition temperature. Furthermore, the d-spacing of the lamellar gel and liquid–crystal phase appeared to be unaffected by the presence of α-tocopherol. The phase behaviour in the mixture was almost identical to that observed in mixtures of DMPC and α-tocopherol [26], and differs markedly from mixtures of DOPE and α-tocopherol (Fig. 2). This is consistent with a model in which α-tocopherol preferentially interacts with either domains of DMPC or domains comprised of DOPE and DMPC that are constrained in a bilayer structure. Phase-separated domains of DOPE and α-tocopherol in H_{II} phase could not be detected in the mixture at least containing up to 10 mol% α-tocopherol.

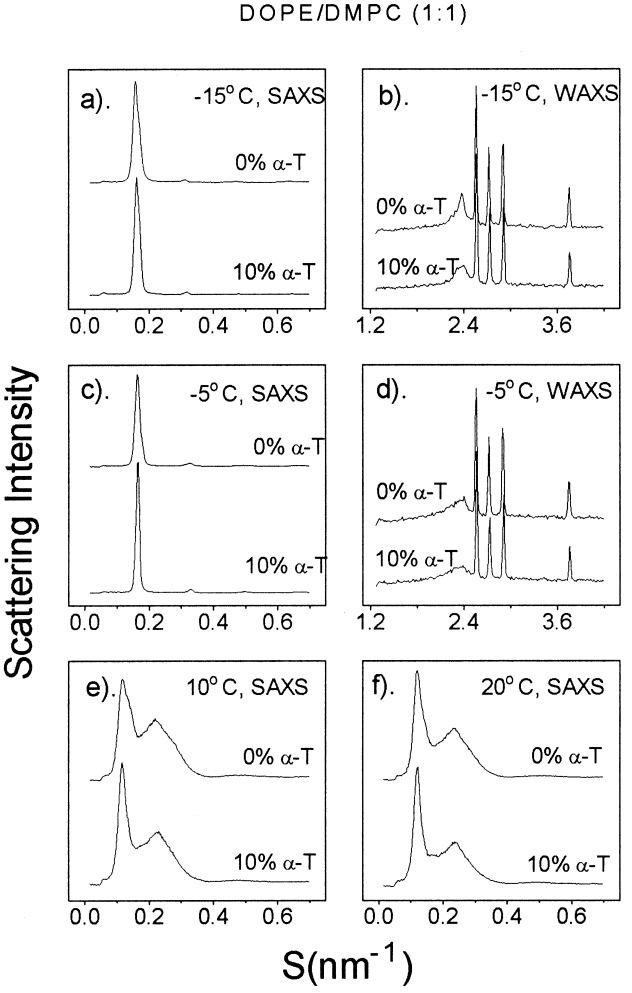

Fig. 9. Static SAXS (a,c,e,f) and WAXS (b,d) intensity profiles as a function of reciprocal spacing of fully hydrated dispersions of DOPE/DMPC (1:1) containing 0 and 10 mol% α-tocopherol recorded at −15°C, −5°C, 10°C and 20°C.

4. Discussion

The effect of α-tocopherol on phase behaviour and structure of aqueous dispersions of saturated PCs and PEs has been reported elsewhere [14, 15,26]. The results obtained showed that the effect of α-tocopherol differed markedly between these two phospholipid classes. Mixtures of α-tocopherol and PC invariably form lamellar phases as does the pure phospholipid but the formation of non-lamellar H_{II} phase was observed in mixtures of α-tocopherol and PE at temperatures below the lamellar gel to liquid–crystal phase transition temperature of the pure phospholipid. The present X-ray diffraction study of mixtures of α-tocopherol with DOPC and DOPE showed that the effect of α-tocopherol on unsaturated phospholipids follows a similar pattern to that observed for their saturated counterparts.

The H_{II} induced by α-tocopherol in PEs can be used as a marker for (PE+α-tocopherol) domain in

PC/PE mixtures. However, in the present X-ray diffraction study of mixed aqueous dispersions of α-tocopherol with DOPE/DOPC (1:1) and DOPE/DMPC (1:1), it was found that lamellar gel and liquid–crystalline phases dominated the phase structure. This is consistent with the fact that α-tocopherol does not preferentially interact with PE. The changes in the SAXS patterns are similar to those of mixtures of α-tocopherol and PCs or of equimolar mixtures of PE/PC. This again suggests that α-tocopherol preferentially interacts with the PC in the mixture or distributes randomly in domains containing both PE and PC regardless of the saturation and the length of hydrocarbon chains of the two phospholipids. The effect of α-tocopherol on mixed dispersions of DOPE/DOPC (3:1) presented a more complex situation. This mixture of phospholipids forms a cogel phase but phase separates into lamellar liquid–crystal and H_{II} phases at temperatures greater than 65°C. The presence of 1, 2 or 5 mol% α-tocopherol caused a decrease in the temperature at which the H_{II} appears. Phase separation of non-lamellar phase from L_β can be detected in the presence of 7.5 and 10 mol% α-tocopherol, indicating a limited capacity of the DOPC to complex with α-tocopherol in the lamellar domain.

The conclusions drawn from the X-ray data presented here appear to differ from interpretation of the effect of α-tocopherol on PE/PC mixtures using DSC reported by Ortiz et al. [18]. These studies obtained calorimetric evidence that indicated that there was a preferential partition of α-tocopherol into the lowest melting component of PC/PE mixtures irrespective of whether this was PC or PE. This conclusion also does not seem consistent with the considerable data indicating formation of stoichiometric complexes between α-tocopherol and PCs.

Although α-tocopherol can be regarded as a relatively minor component of biological membranes, its tendency to form complexes with membrane lipids suggests that it may be localised in domains within the membrane. This non-random distribution in the plane of the membrane may have consequences with regard to its antioxidant or membrane stabilising function but it is clear that the structure and stability of α-tocopherol rich domains would have defined characteristics. The present study of the effect of α-tocopherol on model membranes supports the idea that α-tocopherol does not have a preferred interaction with unsaturated PE when codispersed with either saturated or unsaturated molecular species of PC. Whether this is due to formation of complexes between α-tocopherol and PCs has yet to be determined.

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