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A differential scanning calorimetry study of the interaction of α-tocopherol with mixtures of phospholipids

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(1) When α-tocopherol was included in multibilayer vesicles of dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine it induced a broadening of the main transition and a displacement of this transition to lower temperatures, as seen by differential scanning calorimetry. This effect was quantitatively more important in the samples of distearoylphosphatidylcholine than in those of the other phosphatidylcholines. (2) α-Tocopherol when present in equimolar mixtures of dimyristoylphosphatidylcholine and diastearoylphosphatidylcholine, which show monotectic behaviour, preferentially partitions in the most fluid phase. (3) The effect of α-tocopherol on the phase transition of dilauroylphosphatidylethanolamine and dipalmitoylphosphatidylethanolamine is qualitatively different of that observed on phosphatidylcholines, and several peaks are observed in the calorimetric profile, probably indicating the formation of separated phases with different contents in α-tocopherol. The effect was more apparent in dipalmitoylphosphatidylethanolamine than in dilauroylphosphatidylethanolamine. (4) The inclusion of α-tocopherol in equimolar mixtures of dilauroylphosphatidylethanolamine and dipalmitoylphosphatidylcholine, which show cocrystallization, only produced a broadening of the phase transition and a shift to lower temperatures. However, in the case of equimolar mixtures of dipalmitoylphosphatidylcholine which also show cocrystallization, the effect was to cause lateral phase separation with the formation of different mixtures of phospholipids and α-tocopherol. (5) α-Tocopherol was also included in equimolar mixtures of phosphatidylethanolamine and phosphatidylcholine showing monotectic behaviour, and in this case α-tocopherol preferentially partitioned in the most fluid phase, independently of whether this was composed mainly of phosphatidylcholine or of phosphatidylethanolamine.

Introduction

α-Tocopherol is a very important component of biological membranes which in animals is predominantly found in the membranes of subcellular organelles and also in the plasma membrane.

α-Tocopherol is a membrane-stabilizing agent [1]. This stabilization may be produced through inhibition of the peroxidation of membrane lipids [2,3], by forming complexes with potentially toxic unsaturated fatty acids [4] or by restricting the

Abbreviations: DSC, differential scanning calorimetry; T_m, midpoint of the gel to liquid-crystalline transition; ΔH, total enthalpy change of the transition; PC, diacylphosphatidylcholine; PE, diacylphosphatidylethanolamine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DLPE, dilauroylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; Mops, morpholinepropanesulfonic acid.

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molecular mobility of the membrane components [5,6].

A number of physical techniques, including DSC [7–10], ESR [11], fluorescence [12], ^{2}H-NMR [9] and Fourier transform-infrared spectroscopy [10], have been used to demonstrate that increasing concentrations of α-tocopherol progressively broaden the temperature range of the phase transition with its onset temperature lowered, and the enthalpy of the gel to liquid-crystalline transition is reduced in bilayers of fully saturated phospholipids.

Most of these studies were performed with phosphatidylcholines (PC). But given the heterogeneous phospholipid composition of biological membranes it must be interesting to know whether α-tocopherol shows a similar effect on phospholipids different of PC. In this paper we present our studies, using DSC, of the interaction of α-tocopherol with different phosphatidylcholines and phosphatidylethanolamines and the possible preference of α-tocopherol for interacting with any of them.

Materials and Methods

Dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine were obtained from Fluka, Buchs, Switzerland. Distearoylphosphatidylcholine and α-tocopherol from Sigma, Poole, Dorset, U.K. All the others phospholipids were from Avanti Polar Lipids, Birmingham, AL, U.S.A.

The lipid mixtures for microcalorimetry measurements were prepared by combination of chloroform solutions containing 2.72 μmol of each phospholipid and the appropiate amount of α-tocopherol when indicated, giving a final volume of 50–200 μl of chloroform in a small tube of 8 mm (inner diameter). The organic solvent were evaporated under a stream of N_2, at a temperature above the highest transition temperature of the lipids present and the last traces of solvent were removed by a further 3–5 h evaporation under vacuum. After the addition of 50 μl of twice-distilled and deionized water, multilamellar liposomes were formed by mixing using a bench vibrator (Super-mixer, Lab-Line Instruments Inc. Melrose Park, IL, U.S.A.) at the maximum speed and keeping the samples at a temperature above the highest T_c of the mixture. Mixing was continued until a homogeneous and uniform suspension was obtained. The longest period was necessary for samples wich contained the highest proportion of α-tocopherol.

Alternatively, some samples were dispersed in 10 mM Mops (pH 7.0). 15-μl aliquots of these suspensions containing 0.81 μmol of each lipid were sealed in small aluminium pans and scanned in a Perkin-Elmer DSC-4 calorimeter, using a reference pan containing water. The heating rate was 4 K/min in most of the samples, although some of them were scanned at 0.5 K/min. Peak areas were measured by weighing paper cut-outs of the peaks. The instrument was calibrated using indium as standard.

Results

The effect of α-tocopherol on saturated phosphatidylcholines

The effect of α-tocopherol on the thermotropic phase transition of DMPC, DPPC and DSPC has been studied using DSC. Fig. 1 shows that concentrations of α-tocopherol as low as 2 mol% already introduce a significant perturbation in these phospholipids. The pretransition was abolished and the main transition broadened and shifted to lower temperatures. The main transition totally disappeared at 40 mol% with the three phospholipids. Fig. 2 shows that in the presence of

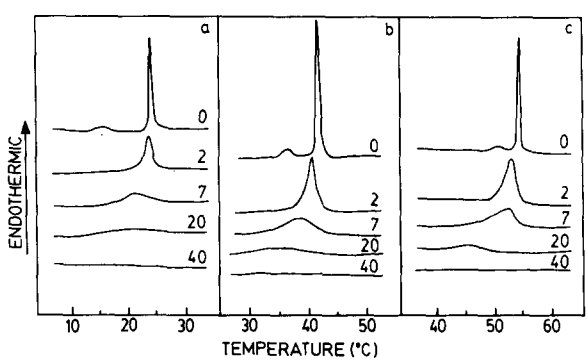

Fig. 1. The DSC calorimeter curves for pure phosphatidylcholines and phosphatidylcholine/α-tocopherol systems. Molar% contents of α-tocopherol are indicated on the curves. The curves correspond to the same amount of phospholipid in each case. Panel (a) DMPC-containing samples; (b) DPPC-containing samples; (c) DSPC-containing samples.

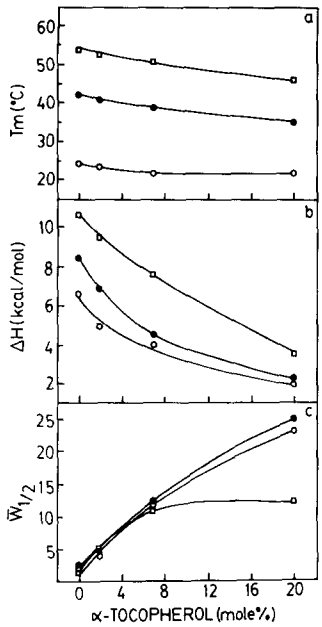

Fig. 2. Changes in midpoint of the transition temperature (T_m), total enthalphy (ΔH) and half-height width($W_{1/2}$), for mixtures of α-tocopherol containing (○) DMPC, (●) DPPC and (□) DSPC.

α-tocopherol, T_m was decreased for the three phospholipids, as well as ΔH of the main transition, the decrease in ΔH being not a linear function of α-tocopherol content. On the other hand the width of the transition peak at half-maximum height was increased, although it is appreciated in Fig. 2c that DMPC and DPPC are more affected than DSPC at high concentrations of α-tocopherol. This may indicate that α-tocopherol disrupts the cooperative behaviour of the lipid bilayer matrix.

Mixtures of α-tocopherol with two different species of phosphatidylcholine

The effect of α-tocopherol upon the thermotropic behaviour of mixtures of various phosphatidylcholines can give us information as to whether α-tocopherol interacts randomly or preferentially with one of the molecular species of PC present. We have examined both mixtures in which the two molecular species of PC are miscible above and below the order-disorder transition, and mixtures where phase separation occurs.

It is known that DSPC and DPPC show cocrystallization of the paraffin chains [13,14]. Fig. 3a illustrates that the single peak detected for the transition of an equimolar DPPC/DSPC mixture was modified by the presence of increasing concentrations of α-tocopherol in a qualitatively similar manner to these phospholipids in separate, so that there is a shift in the transition towards lower temperatures and an increase in the width of the peak. No phase separation was induced by α-tocopherol and no indication of any preference of interaction of α-tocopherol with any of these phospholipids may be inferred.

DMPC and DSPC, on the other hand, are known to show monotectic behaviour [13,14]. Fig. 3b shows how increasing concentrations of α-tocopherol preferentially affects the lower melting component which corresponds to DMPC. The incompletion of the phase separation makes it difficult to give a quantitative description of the phenomenon, but it is clearly seen that whereas the highest component was broadened and shifted, the lowest component had already disappeared at 20 mol%. It is also interesting that the higher component was still present at 40 mol% whereas this concentration totally suppressed the transition in pure DSPC (Fig. 1a).

Fig. 4 shows how the total ΔH was decreased by increasing concentrations of α-tocopherol, in a non-linear manner.

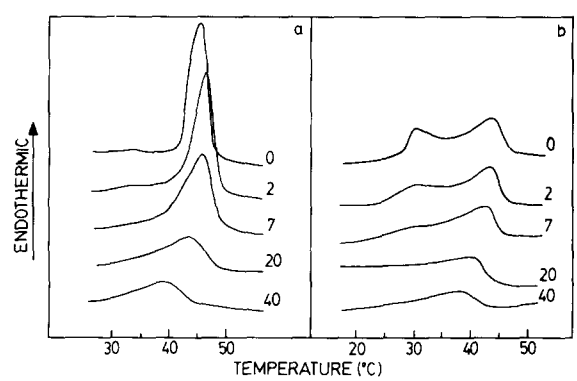

Fig. 3. The DSC calorimetric curves for mixtures of DPPC/DSPC (a) and DMPC/DSPC (b) containing different amounts of α-tocopherol. Molar% contents in α-tocopherol are indicated on the curves. All the samples contain equimolar amounts of both phospholipids.

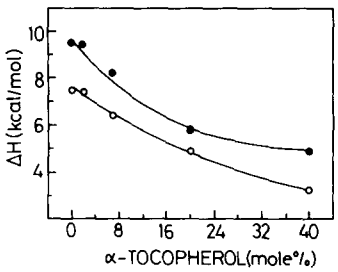

Fig. 4. Changes in ΔH with α-tocopherol content for the systems described in Fig. 3. (○) DMPC/DSPC and (●) DPPC/DSPC.

The effect of α-tocopherol on DLPE and DPPE

α-Tocopherol considerably affected the phase transition of these saturated phosphatidylethanolamines, and the effect was qualitatively different from that observed on phosphatidylcholines. Fig. 5a shows that the phase transition of DLPE was shifted to lower temperatures and at 7 mol% of α-tocopherol two peaks were observed. At 20 mol% only one peak remained and at 40 mol% the transition was totally eliminated. The pattern observed in DPPE/α-tocopherol mixtures was rather complex (Fig. 5b), showing two peaks already at 2 mol% of α-tocopherol and even three at 7 mol%. At 20 mol% only two peaks remained and at 40 mol% the transition was totally inhibited.

It seems that α-tocopherol does not give a good mixing with these phosphatidylethanolamines and lateral phase separations occur, probably producing phases with different contents in α-tocopherol and the phospholipid, so that the transition temperature will be lower as more α-tocopherol is present in each particular phase.

The results described above were obtained with samples prepared in twice-distilled water. Since pH affects the transition temperature of phosphatidylethanolamine bilayers [15] some samples of pure DPPE and DPPE containing 20 mol% of α-tocopherol were prepared in pH 7.0 buffer and the results obtained were exactly the same than those found with samples prepared in water.

Our samples were prepared always at temperatures above T_m and care was taken in obtaining a uniform dispersion and a good hydration, so that in fact the scans shown above were perfectly reversible and the pattern obtained when the scans were repeated were always identical to the first one.

On the other hand, two different scan rates were used for some samples. 4 K/min and 0.5 K/min, and the patterns were the same in both cases.

Fig. 6 shows that the total ΔH decreased as more α-tocopherol is incorporated in the mixtures with both DPPE and DLPE.

Effect of α-tocopherol on mixtures of phosphatidylcholines and phosphatidylethanolamines

In order to investigate the possible preference of α-tocopherol for PC or PE which are two of the most common phospholipids in biological membranes, α-tocopherol was incorporated in equi-

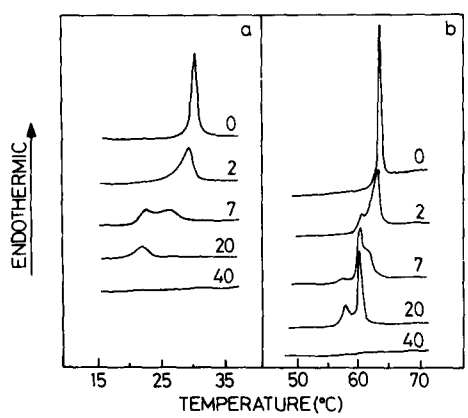

Fig. 5. The DSC calorimetric curves for pure DLPE(a) and pure DPPE(b) containing different amounts of α-tocopherol. Molar% contents in α-tocopherol are indicated on the curves.

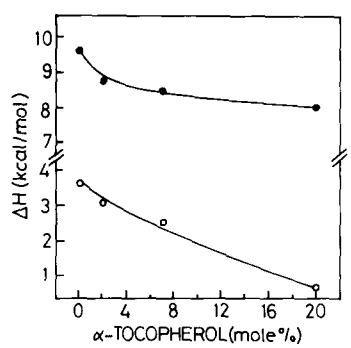

Fig. 6. Changes in ΔH with α-tocopherol content for the systems described in Fig. 5. (○) DLPE and (●) DPPE.

molar mixtures showing either cocrystallization or phase separation.

DLPE/DPPC and DPPC/DPPE are mixtures which show cocrystallization and a single peak was obtained in DSC, situated at temperatures which are intermediate between those where pure phospholipids undergo the phase transition. Note that these two couples were selected because in DLPE/DPPC the phosphatidylethanolamine has the lowest T_c whereas in DPPC/DPPE this occurs to the phosphatidylcholine.

DLPE/DPPC mixture yielded a fairly narrow peak with T_m at 35°C (Fig. 7a). When increasing concentrations of α-tocopherol were incorporated into this mixture, a broadening of the peak and a shift to lower temperatures was observed. The transition peak was very smeared out at 20 mol% of α-tocopherol with T_m at 26.3°C. At 40 mol% a very broad transition was still observed.

DPPC/DPPE mixtures have been found to give only limited solid phase miscibility [16], and in fact the equimolar mixture showed a broad transition peak (Fig. 7b). It is shown in this figure that α-tocopherol induces a destabilization of the DPPC/DPPE mixture giving place to the appearence of more than one peak. At 2 mol% a smaller peak is seen at a temperature below the main T_m, and at 7 mol% even two peaks are visible.

It is interesting that at 40 mol% the transition is not fully eliminated, as it was described above for the pure phospholipids. It should be observed that none of these peaks correspond to mixtures of a pure phospholipid with α-tocopherol since the temperature at which they appear are too high to correspond to a DPPC/α-tocopherol mixture or too low for a DPPE/α-tocopherol mixture, and they do not show a high cooperativity so that they do not seem to arise either from pure phospholipids without α-tocopherol.

Fig. 8 shows that ΔH was decreased as more α-tocopherol was included in the mixtures. There was a non-linear dependence, and it is interesting that the transition was not totally suppressed at 40 mol% of α-tocopherol which is at variance with the observations made for either of the pure phospholipids containing the same concentration of α-tocopherol.

In another set of experiments, α-tocopherol was included in equimolar mixtures of phosphatidylethanolamines and phosphatidylcholines which showed solid phase separation. However, the phase separation is not complete, and thus the two peaks observed are broader than those of the pure phospholipids (Fig. 9a), and shifted to a higher temperature in the case of DMPC (T_m = 26.6°C, compared to 23°C for pure DMPC) and to a lower temperature in the case of DPPE (T_m = 52°C, compared to 63.8°C for pure DPPE).

The inclusion of 2 mol% of α-tocopherol produced a shift to a higher temperature of the peak rich in DMPC (T_m = 32.5°C), and since this behaviour was not found for pure DMPC, where α-tocopherol produced a decrease in T_m, the explanation must be that 2 mol% of α-tocopherol favored the formation of a mixture richer in DPPE

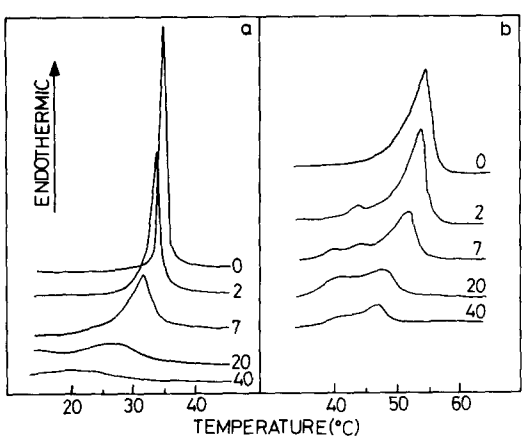

Fig. 7. The DSC calorimetric curves for mixtures of DLPE/DPPC(a) and DPPC/DPPE(b) containing different amount of α-tocopherol. Molar% contents in α-tocopherol are indicated on the curves.

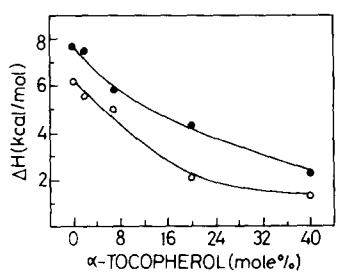

Fig. 8. Changes in ΔH with α-tocopherol content for the systems described in Fig. 7. (○) DLPE/DPPC and (●) DPPC/DPPE.

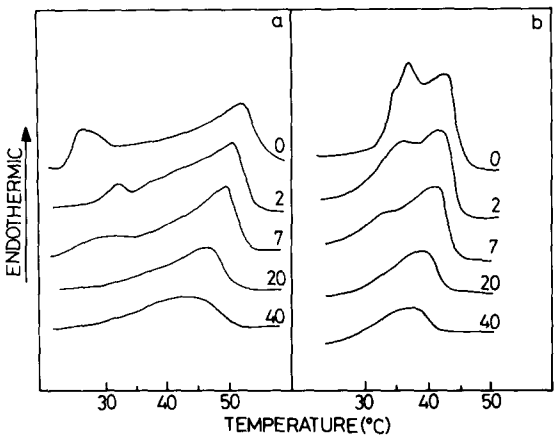

Fig. 9. The DSC calorimetric curves for mixtures of DMPC/DPPE(a) and DLPE/DSPC(b) containing different amounts of α-tocopherol. Molar% contents in α-tocopherol are indicated on the curves.

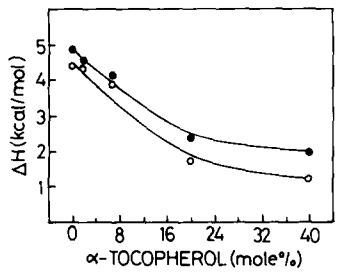

Fig. 10. Changes in ΔH with α-tocopherol content for the systems described in Fig. 9. (○) DLPE/DSPC and (●) DMPC/DPPE.

which was responsible for the observed shift in T_m. On the other hand the peak rich in DPPE was broadened at this concentration of α-tocopherol, and T_m decreased. At 7 mol% of α-tocopherol both peaks were broadened with respect to the sample with 2 mol%, and T_m decreased in both peaks. It seems that the effect of α-tocopherol on the cooperativity of the phospholipids was now more important than its mixing effect observed in the 2 mol% sample. At 20 mol% the lowest component was not any longer observed and the higher one was again broadened and shifted to lower temperatures. A concentration of 40 mol% of α-tocopherol produced a single and broad peak centered at 43.5°C.

In the case of the DLPE/DSPC mixture (Fig. 9b) the lower melting component was now the PE. Again an incomplete phase separation was observed, with two peaks having T_m at 37.2°C and 42.6°C, i.e. at higher temperature than pure DLPE ($T_m = 30.2$°C) in the case of the lowest melting component, and at lower temperature than pure DSPC ($T_m = 54.5$°C) in the case of the highest melting component. Increasing concentrations of α-tocopherol produced here the disappearance of the lowest melting component at 20 mol% of α-tocopherol, so that only one peak remained in this mixture, centered at 38.9°C.

Finally, Fig. 10 shows the changes in total ΔH of the samples as more α-tocopherol was incorporated into the phospholipid mixtures. ΔH decreases with α-tocopherol content, but there was a non-linear dependence as in the other cases examined above. It is also interesting that at 40 mol% the transition is still present, and this is at variance with the results obtained for any of the pure phospholipids when mixed with the same concentration of α-tocopherol.

Discussion

Interaction of α-tocopherol with phosphatidylcholines

The differential scanning calorimetry measurements of mixtures of α-tocopherol with three different saturated phosphatidylcholines showed that the effect of α-tocopherol on the thermotropic behaviour of these phospholipids is qualitatively similar, although at high concentrations of α-tocopherol the cooperative behaviour of DSPC seems to be less disrupted than those of DMPC and DPPC. The effect of α-tocopherol on this transition is to decrease ΔH and T_m and to increase the width of the peak. The same behaviour was observed in a mixture of DPPC/DSPC which shows cocrystallization.

The direction of the shifts in the midpoint of the phase transition curve (T_m) has been used to determine whether a free fatty acid partitions into gel or fluid phase lipid, using ideal solution theory [17]. According to that, if T_m decreases in the presence of a solute then this solute tends to partition in the fluid phase. Supposing that this is applicable to α-tocopherol, then just from its ef-

fect on single phosphatidylcholines of decreasing their T_m, it can be concluded that α-tocopherol has a preference to partition into fluid phases. The same can be concluded from the experiments done using a mixture of DMPC/DSPC where a monotectic behaviour is observed, and α-tocopherol preferentially affect the lower melting component. It is then concluded that α-tocopherol interacts randomly with phosphatidylcholine species when there is cocrystallization of their paraffin chains, but α-tocopherol will be distributed in a non-random form at temperatures at which phase separation occurs.

This behaviour of α-tocopherol is similar to that previously observed for cholesterol [14] which is another extremely important membrane component which similarly to α-tocopherol seems to stabilize phospholipid bilayers.

It is also interesting to compare the effect of α-tocopherol with other long-chain amphiphilic molecules. Long-chain saturated or *trans*-unsaturated free fatty acids and fatty alcohols have been shown by a variety of physical techniques to increase and broaden the main gel to liquid-crystalline phase transition of saturated phosphatidylcholine bilayers whereas fatty acids or fatty alcohols and fatty acid derivative probes lower and broaden the transition, all of these molecules being able of eliminating the pretransition [18–21]. It has been suggested also [17] that *cis*-unsaturated fatty acids partition into fluid domains while the *trans*-unsaturated and saturated fatty acids preferentially partition into solid-like domains. It is evident that α-tocopherol behaves more like *cis*-unsaturated fatty acids. *cis*-Unsaturated fatty acids are expected to decrease the van der Waals interactions between the terminal methyl and methylene groups of the phospholipid hydrocarbon chains, and this will lower the transition temperature [18]. Certainly α-tocopherol could produce this type of effect because of its methyl substituents in the phytyl side chain.

On the other hand it is worthwhile also to compare the effect of α-tocopherol with that of other similar molecules like ubiquinone-3 which also induces a broadening and decrease of the phase transition of DPPC [22] and vitamin K_1 which apart from causing also a broadening and decrease of the phase transition of DPPC originates a second phase at lower temperatures when high concentrations are included in the membrane [23]. Thus the effect of α-tocopherol is similar to these molecules in broadening and decreasing the phase transition of DPPC and it is interesting that high concentrations of vitamin K_1 in DPPC bilayers induce lateral phase separation as α-tocopherol is shown here to do when mixtured with DPPE or DLPE (Fig. 5).

In any case we have shown before [10] that α-tocopherol may perturb the interfacial region of DPPC bilayers whereas α-tocopheryl acetate, which was not able of doing so, has a qualitatively weaker effect. Therefore we suggest that both the phytyl side chain and the chromanol ring may be responsible of the observed effects.

It is noteworthy that the phase transition is not totally eliminated at 40 mol% o α-tocopherol in mixtures of different diacylphosphatidylcholines, although this concentration totally suppressed the transition of any of these phospholipids in separate. The reason must be that α-tocopherol is totally excluded from the phase which is still undergoing the transition.

α-Tocopherol induced a decrease in T_m as well, when included in multibilayers of other phospholipids, such as DPPG, egg yolk sphingomyelin (which contains about 80% of palmitoyl) (results not shown), and also DPPE and DLPE as shown below. Then this behaviour of α-tocopherol seems to be common with respect to multibilayer vesicles made of a wide variety of phospholipid types.

Interaction of α-tocopherol with phosphatidylethanolamines

The effect of α-tocopherol on the phase transition of the PE's is qualitatively different of that described above for PC's. As seen in Fig. 5, different phases seem to be formed when α-tocopherol is present, although the total ΔH and the transition temperatures are progressively decreased similarly to the α-tocopherol/PC mixtures.

A more complex behaviour was observed for DPPE than for DLPE. Although the reason is not clear, it might be related to the length of the fatty acyl chains.

It has been described that saturated phosphatidylethanolamines show metastability when samples are prepared at temperatures below the

gel to liquid-crystalline phase transition [24,25] or prepared at temperatures above this transition but cooled down afterwards at 2–4° C and kept chilled for several days or even weeks [16,25–27]. In all the cases new phases have been observed by DSC but only during the first scan, since once the samples have been heated above the T_m and cooled down again only one phase transition remains and the T_m of this phase transition is the same as that observed in samples prepared directly at temperatures above T_m. It is also a common finding of all the references mentioned above that the new phases have melting temperatures higher than the characteristic T_m of the gel to liquid-crystalline phase transition.

The metastability has been attributed to low hydration of the samples when they are prepared below T_m or kept chilled for long periods of time [24–27] so that the higher temperature transitions would arise from the simultaneous hydration and acyl chain melting of these saturated phosphatidylethanolamines [24].

It is shown in this paper that α-tocopherol induces the appearance of more than one phase and this might be thought to be related with the lyotropic polymorphism of the saturated phosphatidylethanolamines. However we find this possibility unlikely, first in all because our samples have been carefully prepared to ensure a good hydration and apart from that we find that all the phases arising from the effect of α-tocopherol have transition temperatures which are lower than that of the gel to liquid-crystalline phase transition of the pure phospholipid in contrast with the pattern observed with poorly hydrated samples, where the new phases have higher transition temperatures. As discussed above the decrease in T_m induced by α-tocopherol seems to be common to a variety of phospholipids.

The effect of α-tocopherol on the PE's may be due to the perturbation of the intermolecular hydrogen bonds present in phospholipid vesicles made of this phospholipid [28].

Interaction of α-tocopherol with PE / PC mixtures

The behaviour of α-tocopherol in PE/PC mixtures is also very interesting. When included in mixtures showing monotectic behaviour such as DLPE/DSPC and DMPC/DPPE, α-tocopherol preferentially interacts with the lowest melting component independently of whether this is a PC or a PE. 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 [29].

In the case of the DLPE/DPPC sample, the pattern found (Fig. 7a) was that α-tocopherol induced a broadening of the narrow peak observed for the mixture of phospholipids with a shift of the phase transition to lower temperatures, but there was not any indication of a phase separation induced by α-tocopherol and hence it seems that α-tocopherol has no preferential affinity for DPPC, but just partition into the most fluid phase during the transition, and hence it makes T_m to decrease [17].

On the other hand, a more complex pattern was found in the DPPC/DPPE mixture, where the peak was wider than in the DLPE/DPPC case, possibly indicating a less ideal mixing. In the DPPC/DPPE samples, α-tocopherol induced the appearance of several peaks at temperatures lower than that of the main one. As indicated above these peaks seem to arise from mixtures of varying composition in phospholipids and possibly also in α-tocopherol, and they may be reminiscent of the complex pattern observed for DPPE/α-tocopherol mixtures (Fig. 5b). It seems again evident that α-tocopherol tends to partition in fluid phases and hence as α-tocopherol content increased transition peaks were observed at lower temperatures.

In conclusion it is not possible to infer from these observations that α-tocopherol had a preferential affinity for either PC or PE phospholipids, but just that it tends to partition into the most fluid phases, and also that it interacts in a different way with PE's, specially with DPPE, being able of producing lateral phase separations when DPPE was present.

Biological implications

The existence of lipid domains giving lipid heterogeneity in the lateral plane of the bilayer has been postulated for a number of biological membranes, including animal and plant plasma membranes [17,30–32]. 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 [33] and differences in fluidity between bilayers halves of plasma membranes is another claim supported by certain experiments [34–36].

As a consequence of these lateral and transverse asymmetries, α-tocopherol will not be homogeneously distributed in the membrane, but rather associated to the most fluid zones. Incidentally this will cause α-tocopherol to be associated to more unsaturated fatty acyl chains and hence will facilitate its peroxidation-protecting task.

On the other hand, α-tocopherol may cause structural alterations in the membrane when PE's are present, since it seems to be able of disrupting the interactions between PC's and PE's, but, given the low concentration of α-tocopherol in biological membranes, this might be less relevant in order to influence the structure of membranes in vivo.

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