Biochimica et Biophysica Acta, 1022 (1990) 194–202
Elsevier

BBAMEM 74753

Influence of vitamin E on phosphatidylethanolamine lipid polymorphism

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(Received 25 July 1989)
(Revised manuscript received 27 October 1989)

Key words: α-Tocopherol; α-Tocopheryl acetate; DSC; NMR, ^{31}P-; Hexagonal H_{II} phase; Phosphatidylethanolamine

The effect of vitamin E, in its major form α-tocopherol and its synthetic analog α-tocopheryl acetate, on phosphatidylethanolamine lipid polymorphism has been studied by mean of differential scanning calorimetry and ^{31}P-nuclear magnetic resonance techniques. From the interaction of these tocopherols with dielaidoylphosphatidylethanolamine it is concluded that both molecules promote the formation of the hexagonal H_{II} phase at temperatures lower than those of the pure phospholipid. When the tocopherols were incorporated in the saturated dimiristoylphosphatidylethanolamine, which has been shown not to undergo bilayer to hexagonal H_{II} phase transition, up to 90°C, they induce the phospholipid to partially organize in hexagonal H_{II} phase. From our experiments it is shown that α-tocopherol is more effective than its analog in promoting H_{II} phase in these systems. It is also shown that, while α-tocopheryl acetate does not significantly perturb the gel to liquid-crystalline phase transition of dimirystoylphosphatidylethanolamine, α-tocopherol does so and more than one peak appears in the calorimetric profile, indicating that lateral phase separations are taking place.

Introduction

Vitamin E is known to function in vivo as an antioxidant agent, preventing peroxidation of unsaturated fatty acids present in membrane phospholipids [1]. The most active and most abundant compound with vitamin E activity is α-tocopherol [1]. α-Tocopherol has been widely used, together with its synthetic derivative α-tocopheryl acetate, in the therapy of conditions such as sterility [2], muscular dystrophy [3], anemia [4], etc. α-Tocopherol has also a stabilizing effect on membranes [5], either by forming complexes with potentially toxic unsaturated fatty acids or by restricting the molecular mobility of the membrane components [7,8]. Although the biological function of α-tocopherol is well known, the knowledge of the molecular mechanism of its action is rather limited.

In order to better understand the function of α-tocopherol at the molecular level, it is important to study its interaction with membrane components, and specifically with lipids. A number of physical techniques, including differential scanning calorimetry [9–12], electron spin resonance [13,14], nuclear magnetic resonance [12,15], fluorescence [16–18] and Fourier transform infrared spectroscopy [10] has been used to ascertain the location of α-tocopherol in the membrane and its interaction with phospholipids. These studies concluded that α-tocopherol has its phenolic group located near the polar moiety of the lipid matrix and that increasing concentrations of α-tocopherol progressively broaden the temperature range of the gel to liquid-crystalline phase transition. In bilayers of fully saturated phosphatidylcholines, α-tocopherol lowers the onset temperature and reduces the enthalpy of the phase transition. It has been shown that when incorporated in model membranes with different phospholipid composition, α-tocopherol preferentially partitions into the most fluid domain [11]. Recently, it has been also shown by using the intrinsic fluorescence of the molecule that the chromanol moiety of α-tocopherol is located in a position

Abbreviations: DSC, differential scanning calorimetry; PE, phosphatidylethanolamine; DEPE, dielaidoylphosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; Mops, morpholinepropanesulfonic acid; T_c, onset temperature of the gel to liquid-crystalline phase transition. T_m, midpoint temperature of the gel to liquid-crystalline phase transition. T_H, onset temperature of the lamellar to hexagonal H_{II} phase transition.

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close to that occupied by the probes 7-AS and 5-NS in the membrane and that α-tocopherol has a very high lateral diffusion [19].

It is widely known that dispersions of individual or mixtures of phospholipids of biological origin or synthetic ones can adopt several structures, including the micellar phase, the familiar bilayer phase, the hexagonal H_{II} phase and lipidic particles [20]. The ability of lipids to adopt these different liquid-crystalline structures is known as 'lipid polymorphism'. These non-bilayer structures can greatly affect the functional behaviour of the membrane [21]. They might be intermediates in vesicle fusion, they appear to be involved in lipid flip-flop, and might act as carriers for polar compounds. Since lipid polymorphism has such potentially biological importance, it must be interesting to check whether α-tocopherol, which is a very important membrane component, may modulate the lipid polymorphism. To our knowledge, no attempt has been made to characterize the possible effect of α-tocopherol on the macroscopic organization of phospholipids.

It is the aim of this work to study the effect of vitamin E (α-tocopherol and its analog α-tocopheryl acetate) on lipid polymorphism. We chose phosphatidylethanolamine (PE) as a phospholipid model system, since PE is the major phospholipid of eukariotic systems which spontaneously adopts H_{II} phase in the presence of excess aqueous buffer at physiological temperatures. We have used an unsaturated PE (dielaidoylphosphatidylethanolamine, DEPE) and a saturated one (dimiristoylphosphatidylethanolamine, DMPE). The interaction between vitamin E and these PE systems was studied by using DSC and ^{31}P-NMR techniques, the major finding being that vitamin E is a strong hexagonal H_{II}-phase-promoting agent in the systems under study.

Materials and Methods

α-Tocopherol, α-tocopheryl acetate and D_2O (98%) were obtained from Sigma (Poole, Dorset, UK). 1,2-Dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE) and 1,2-dimiristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) were obtained from Avanti Polar Lipids, Birmingham, AL, USA, and judged chromatographically pure by the use of HPTLC. All other compounds were of analytical grade.

Differential scanning calorimetry (DSC)

Samples of 3 μmol of DEPE or DMPE and the appropriate amount of α-tocopherol or α-tocopheryl acetate in chloroform were dried under a stream of N_2 and stored overnight under vacuum to remove the last traces of solvent. The samples were kept for 30 min at 50 °C to hydrate the phospholipid and then were dispersed in 1 ml of 0.1 mM EDTA, 10 mM Mops, 100 mM NaCl buffer (pH 7.4) at the same temperature. Subsequently the samples were spun for 30 min at 5000 rpm. The pellet was then carefully transferred to a small aluminium pan, and measured in a Perkin-Elmer DSC-4 calorimeter using a reference pan containing buffer. The instrument was calibrated using indium as standard. The samples were scanned with a heating rate of 4 C°/min (occasionally a 0.5 C°/min rate was used, giving no better resolution of the thermograms). The range of temperatures studied was from 20°C to 80°C. Successive scans yielded identical thermograms, the second scan was usually used for transition enthalpy calculations. Peak areas were measured by weighing paper cut-outs of the peaks. After the measurements the pans were opened and the samples were dissolved in chloroform/methanol (1 : 1, v/v). After subsequent perchloric acid hydrolysis, the amount of phospholipid originally present in the sample was determined by the method of Bartlett [22].

^{31}P-NMR

60 μmol of DEPE or DMPE and the appropriate amount of α-tocopherol or α-tocopheryl acetate were mixed in a final volume of 300 μl of chloroform in a small tube (5 cm length, 8 mm outer diameter) and evaporated to dryness under a stream of N_2, followed by overnight storage under vacuum to remove last traces of solvent. 60 μl of buffer (0.1 mM EDTA/10 mM Mops/100 mM NaCl (pH 7.4)) was added to the obtained lipid film. The samples were kept for 60 min at 55 °C to hydrate the phospholipid. The samples were centrifuged during 60 min at 5000 rpm to settle the hydrated lipid at the bottom of the tube. The tube was then put inside a conventional 10 mm NMR tube with external D_2O. ^{31}P-NMR spectra were recorded in the Fourier transform mode on a Bruker CXP300 spectrometer (121.46 MHz) equipped with an Aspect 3000 computer. Temperature was controlled to ±0.5°C with a standard Bruker B-VT-1000 variable temperature control unit. All chemical shift values are quoted in part per million (ppm) from pure lysophosphatidylcholine micelles (0 ppm), positive values referring to low-field shifts. All spectra were obtained in the presence of a gated broad-band decoupling (4 W input power during acquisition time) and accumulated free induction decays were obtained from up to 4000 transients. A spectral width of 50 kHz, a memory of 16K data points, a 0.2 s interpulse time and a 80 ° radio frequency pulse, were used. Prior to Fourier transformation an exponential multiplication was applied resulting in a 100 Hz line broadening. After the ^{31}P-NMR measurements had been carried out, pure phospholipid samples were transferred to aluminium pans and scanned by DSC as a purity criterion to check for potential lipid degradation during the experiments. The obtained thermograms were identical to those found for the pure phospholipids before

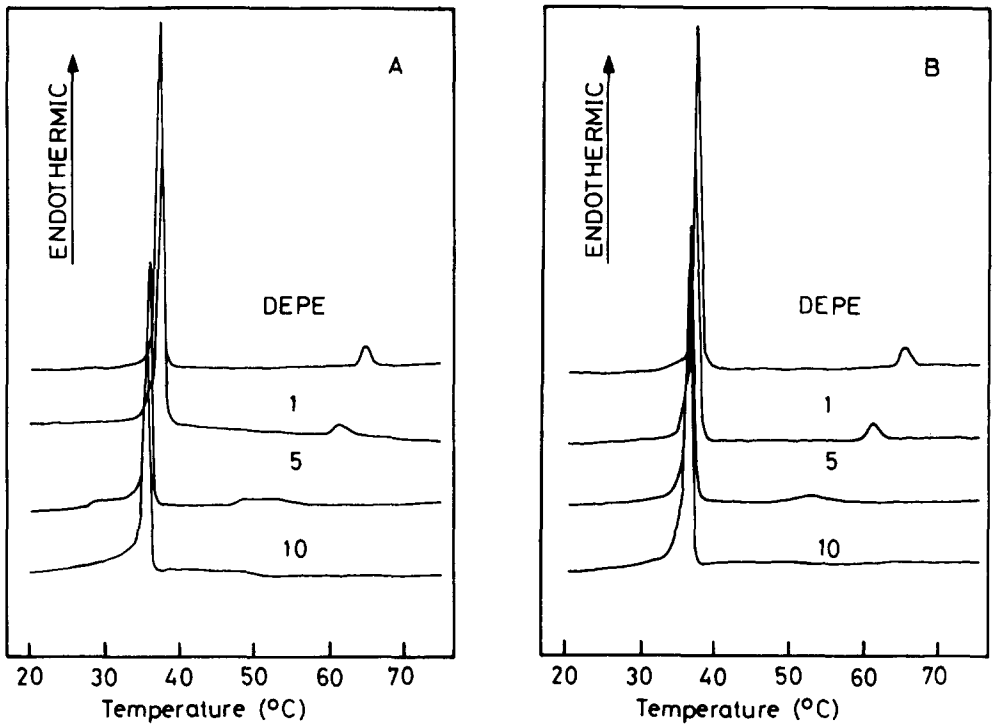

Fig. 1. The DSC thermograms for systems containing pure DEPE, DEPE/α-tocopherol (A) and DEPE/α-tocopheryl acetate (B). Molar percentages of tocopherols in DEPE are indicated on the curves. The curves were normalized for the same amount of phospholipid in each case.

the ^{31}P-NMR measurements, thus indicating that no lipid alterations were produced during the experiments.

Results

Interaction between tocopherols and DEPE

The structural effect of incorporating different amounts of α-tocopherol and its analog α-tocopheryl acetate on DEPE was studied by means of DSC and ^{31}P-NMR. We first studied the effect of vitamin E on the thermotropic phase transitions of DEPE. Fig. 1 shows the calorimetric profile of pure DEPE and mixtures of DEPE/α-tocopherol (Fig. 1A), and DEPE/α-tocopheryl acetate (Fig. 1B). Aqueous dispersions of DEPE can undergo a gel to liquid-crystalline phase transition in the lamellar phase and in addition a lamellar liquid-crystalline to hexagonal H_{II} phase transition [23]. This is shown in the thermogram of pure DEPE dispersed in buffer (Fig. 1, upper part). The gel to liquid-crystalline phase transition occurs around (37 ± 0.5)°C and the bilayer to hexagonal transition occurs around (63 ± 1)°C in agreement with previous data [23], the latter has a much smaller transition enthalpy due to the fluid character of both the lamellar and the hexagonal H_{II} phase [24]. The effect of increasing concentrations of α-tocopherol on DEPE transitions is depicted in Fig. 1A, and it can be seen that the gel to liquid-crystalline chain-melting transition profile is not visibly affected. The effect of α-tocopherol on the bilayer to H_{II} phase transition is more pronounced. Incor-

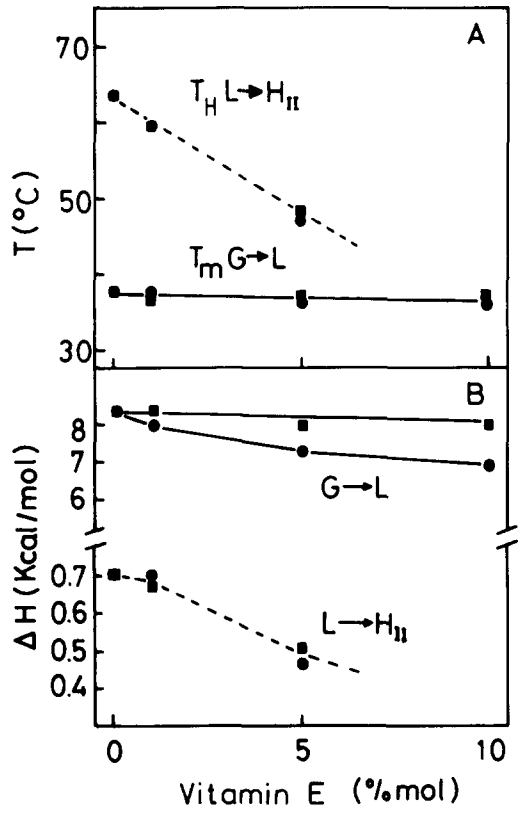

Fig. 2. (A) Changes in the midpoint temperature (T_m) of the gel to liquid-crystalline phase transition (———) and in the onset temperature (T_H) of the lamellar to H_{II} phase transition (— — —). (B) Changes in total enthalpy of the gel to liquid-crystalline (———) and bilayer to H_{II} phase transition (— — —) for DEPE containing α-tocopherol (○) or α-tocopheryl acetate (■) systems.

poration of increasing amounts of the molecule results in a shift of the transition to lower temperatures, and simultaneously, a decrease in area and a broadening of the transition is observed. The comparative effect of α-tocopheryl acetate is shown in Fig. 1B. The effect on the bilayer to H_{II} phase transition is quite similar to that described above for α-tocopherol. The gel to liquid-crystalline transition profile is not affected.

Fig. 2A shows the effect of tocopherols on the gel to liquid-crystalline and bilayer to hexagonal phase transition temperatures of DEPE. For the sharp gel to liquid-crystalline transition, the midpoint temperature (T_c) is shown, and for the broad bilayer to hexagonal transition, the onset temperature (T_H) is shown. Both analogs behave in the same manner respect to the transition temperatures, no significant effect is found on the gel to liquid-crystalline transition temperature with respect to the pure phospholipid. However, the effect on the bilayer to hexagonal transition temperature is clear, a proportional decrease is observed as the tocopherol content increases in the system (actually the transition in the samples with the highest concentration of tocopherols is so broad that it is difficult to calculate the T_H). The effect of tocopherols on the enthalpy of the thermotropic transitions of DEPE is shown in Fig. 2B. For pure DEPE the enthalpies for both transitions were estimated to be 8.3 ± 0.6 kcal/mol for the gel to liquid-crystalline transition and 0.7 ± 0.1 kcal/mol for the bilayer to H_{II} phase transition, in agreement with previous data [23,25]. The presence of α-tocopherol produces a small decrease of the gel to liquid-crystalline transition enthalpy (to 6.9 kcal/mol for the sample with the highest concentration of α-tocopherol), while α-tocopheryl acetate lacks this effect. Both analogs have the same effect on the bilayer to H_{II} transition enthalpy, producing a steepening decrease in the enthalpy.

The effect of tocopherols on the bilayer to H_{II} phase transition was further investigated by means of ^{31}P-NMR. DEPE when organized in bilayer structures give rise to an asymmetrical ^{31}P-NMR lineshape with a

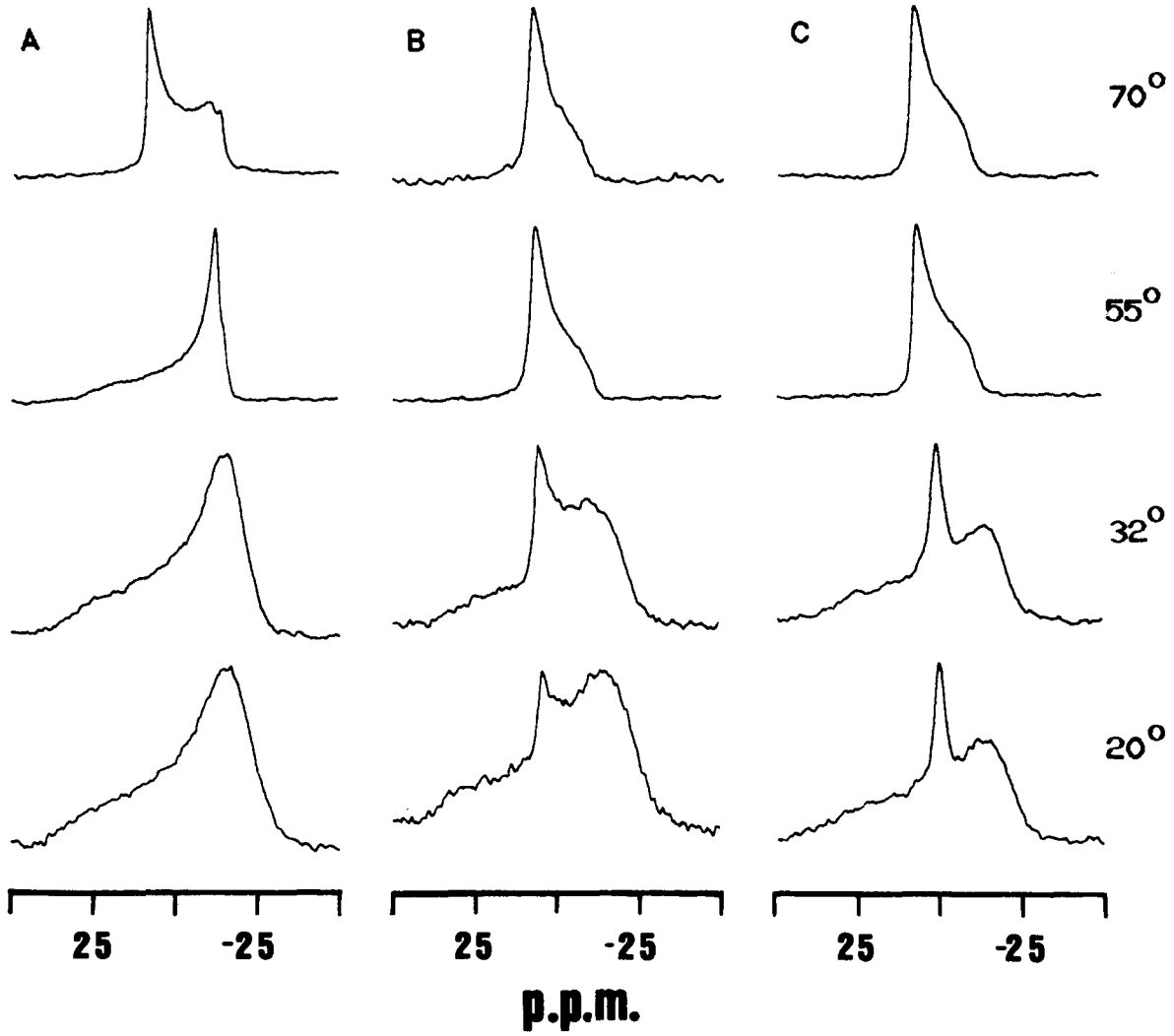

Fig. 3. ^{31}P-NMR spectra of pure DEPE (A) and DEPE containing 10 mol% of α-tocopherol (B) or 10 mol% of α-tocopheryl acetate (C) at different temperatures. The spectra were normalized to the same signal height.

high-field peak and a low-field shoulder [26] and a $\Delta\sigma$ (measured as 3-times the chemical shift difference between the high-field peak and the position of isotropically moving lipid molecules) of approx. 40 ppm, in agreement with previous data [26–28] characteristic of an axially symmetrical shift tensor (Fig. 3A). In the gel state the lineshape is broadened, possibly due to increased ($^1H-^{31}P$) dipolar interactions [29]. In the H_{II} phase, due to rapid lateral diffusion of the phospholipid around the tubes of which this phase is composed, the chemical shift anisotropy is further averaged resulting in a lineshape with a reverse asymmetry, i.e., a high-field shoulder and a low-field peak, with a 2-fold reduction in absolute value of $\Delta\sigma$ [30,31]. When tocopherols were incorporated into DEPE systems the characteristic spectrum corresponding to the hexagonal H_{II} phase appeared at temperatures lower than that of the pure phospholipid (Fig. 3). It is clearly shown that at 55°C, whereas the phospholipid is organized in extended bilayer structures (Fig. 3A), after the addition of α-tocopherol (Fig. 3B) and α-tocopheryl acetate (Fig. 3C) all the phospholipid is organized in hexagonal H_{II} phase. α-Tocopherol give rise to the appearance of hexagonal H_{II} phase at temperatures (20°C) at which the pure phospholipid is organized in the gel bilayer state (Fig. 3B). When the phospholipid is in the gel state, α-tocopheryl acetate gives rise to a spectral component with resonance position at 0 ppm, indicating that the spectrum originating from the DEPE in the gel state is partially replaced, in the presence of α-tocopheryl acetate, by a spectrum characteristic of phospholipid molecules undergoing a rapid motion that leads to a nearly complete averaging of the chemical shift anisotropy.

Interaction between tocopherols and DMPE

The effect of tocopherols on the thermotropic phase transition of DMPE is shown in Fig. 4. Pure DMPE shows only a major endotherm corresponding to the gel to liquid-crystalline bilayer transition and at difference with DEPE, DMPE does not show a bilayer to H_{II} hexagonal phase transition in the range of temperature under study (higher temperatures are difficult to study because the samples are dispersed in aqueous buffer). It was previously reported that DMPE does not present a lamellar to H_{II} phase transition at temperatures below 90°C [32].

The presence of α-tocopherol in DMPE systems produces a rather complex calorimetric profile (Fig. 4A), showing three peaks already at 5 mol% whereas at 20 mol% only two peaks remained. This effect is quite similar to that produced by α-tocopherol on dipalmitoylphosphatidylethanolamine systems as reported before [11]. The effect of α-tocopheryl acetate (Fig. 4B) on DMPE as seen by calorimetry is completely different from that shown above for α-tocopherol. α-Tocopheryl acetate does not significantly perturb the gel to liquid-crystalline transition profile. Surprisingly, a second and

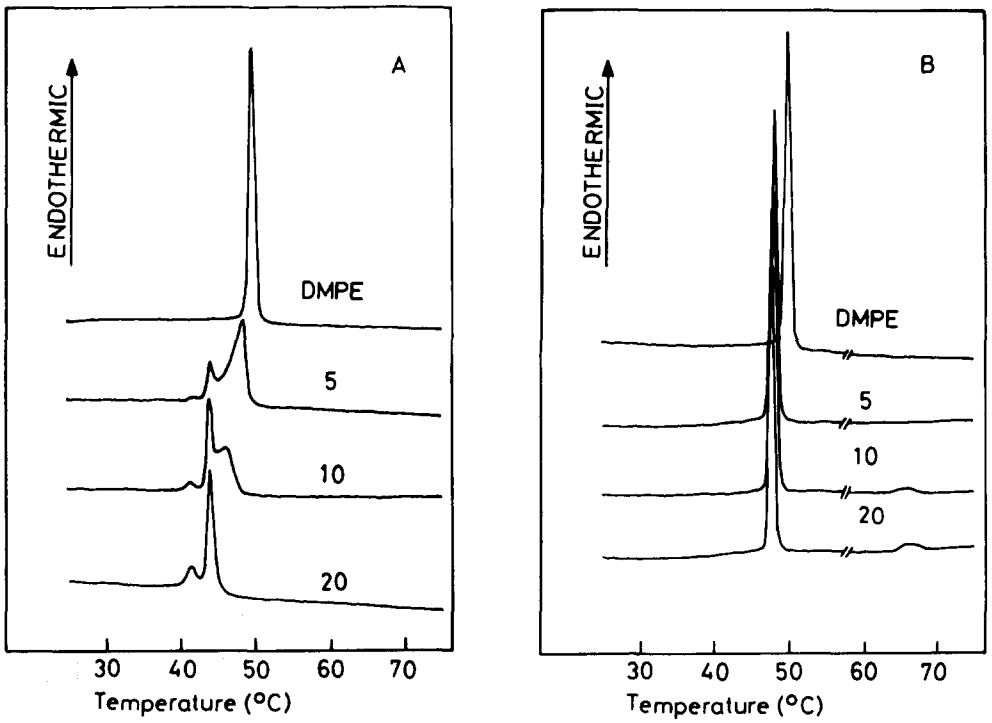

Fig. 4. The DSC thermograms for systems containing pure DMPE, DMPE/α-tocopherol (A) and DMPE/α-tocopheryl acetate (B) The right hand part of the thermograms was recorded at double the sensitivity of left one). Molar percentages of tocopherols in DMPE are indicated on the curves. The curves were normalized for the same amount of phospholipid in each case.

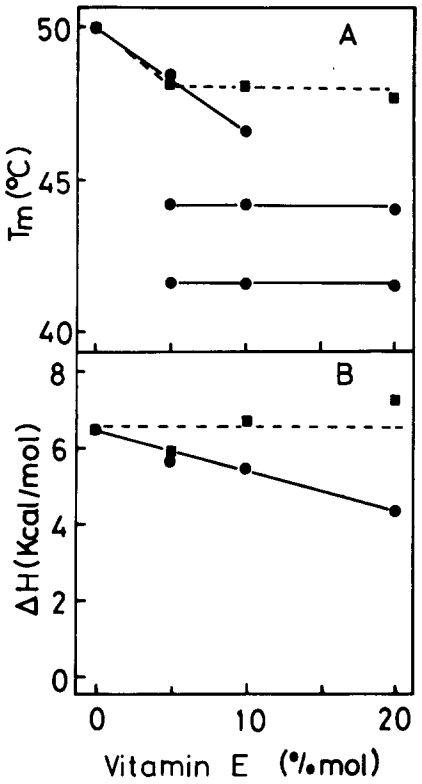

Fig. 5. Changes in midpoint transition temperature, T_m, (A) and total enthalpy, measured as the sum of the different peaks, (B) of the gel to liquid-crystalline phase transition for DMPE containing α-tocopherol (⊙) or α-tocopheryl acetate (■) systems.

very low energy endotherm is detected at higher temperatures, even at 10 mol% of α-tocopheryl acetate. The nature of this second endotherm cannot be discerned from these calorimetric measurements (see below).

Fig. 5A shows the midpoint temperature (T_m) of the gel to liquid-crystalline phase transition for pure DMPE and different DMPE/vitamin E analog systems. It can be seen that, while α-tocopherol produces a decrease of the main transition peak together with the appearance of two new peaks located at approx. (44 ± 0.2)°C and (41 ± 0.1)°C, respectively, the presence of α-tocopheryl acetate has only a slight effect on the transition temperature, since after an initial decrease of approx. 2°C (from (50 ± 0.5)°C for pure DMPE to 48.2°C for 2 mol% of α-tocopheryl acetate) no further changes are observed. Fig. 5B shows the enthalpy of the gel to liquid-crystalline transition for different DMPE/tocopherols calculated as the sum of the three different peaks (ΔH for pure DMPE was estimated to be of 6.4 ± 0.5 kcal/mol). It can be seen that increasing α-tocopherol concentration in the system produces a progressive decrease in the enthalpy of the transition, and that α-tocopheryl acetate has no effect on the enthalpy of this transition (no attempt has been made to calculate the enthalpy of the second endotherm present in the samples with α-tocopheryl acetate due to its very low energy content).

^{31}P-NMR was next used to gain insight into the macroscopic organization which takes place in these systems. The ^{31}P-NMR spectrum of pure DMPE vesicles is shown in Fig. 6A, the familiar asymmetrical lineshape is observed at all temperatures studied indicating that the phospholipid is organized in extended bilayers. The gel to liquid-crystalline transition is evidenced by a decrease in the width of the spectrum and no H_{II} phase transition is detected in the range of temperatures under study. The presence of α-tocopherol (Fig. 6B) gives rise to a new spectral component located at the position characteristic of phospholipid organized in hexagonal H_{II} phase superimposed to a major bilayer signal, which indicates that part of the DMPE molecules, which preferred to organize in bilayer when dispersed alone, are organized in H_{II} phase in presence of α-tocopherol. It can be seen that α-tocopherol induces the appearance of hexagonal H_{II} phase at all temperatures studied except at 30°C, at which an isotropic signal is observed. The results shown above offer an explanation for the pattern found in the calorimetric profiles of these samples where it was not detected any endotherm at temperatures higher than those at which pure DMPE undergo its gel to liquid-crystalline phase transition. The explanation is that some DMPE molecules (most probably those interacting with α-tocopherol) undergo a lamellar to H_{II} phase transition in the same range of temperatures in which the rest of DMPE undergo its gel to liquid-crystalline phase transition.

The effect of α-tocopheryl acetate on DMPE is different from that of α-tocopherol (Fig. 6C), since no changes in the ^{31}P-NMR lineshape are observed at temperatures below the gel to liquid-crystalline transition temperature but, at temperatures above this transition (70°C in Fig. 6C) part of the DMPE molecules are organized in hexagonal H_{II} phase. This agrees with the DSC thermograms of these samples and indicates that the small endotherm detected at higher temperatures (about 65°C) corresponds to a lamellar to H_{II} phase transition of part of the DMPE molecules which is influenced by the presence of α-tococopheryl acetate.

Discussion

In this study, the effect of vitamin E on the macroscopic organization of two different phosphatidylethanolamines has been investigated using α-tocopherol and its analog α-tocopheryl acetate.

Temperature is an important experimental parameter which determines the macroscopic structure of hydrated membrane lipids. Temperature-dependent bilayer to hexagonal H_{II} phase transitions have been observed in a large variety of both synthetic and natural PE's [21]. The bilayer to H_{II} phase transition depends strongly on the fatty acid composition of the lipid. Increasing unsaturation results in decreased bilayer to H_{II} transition

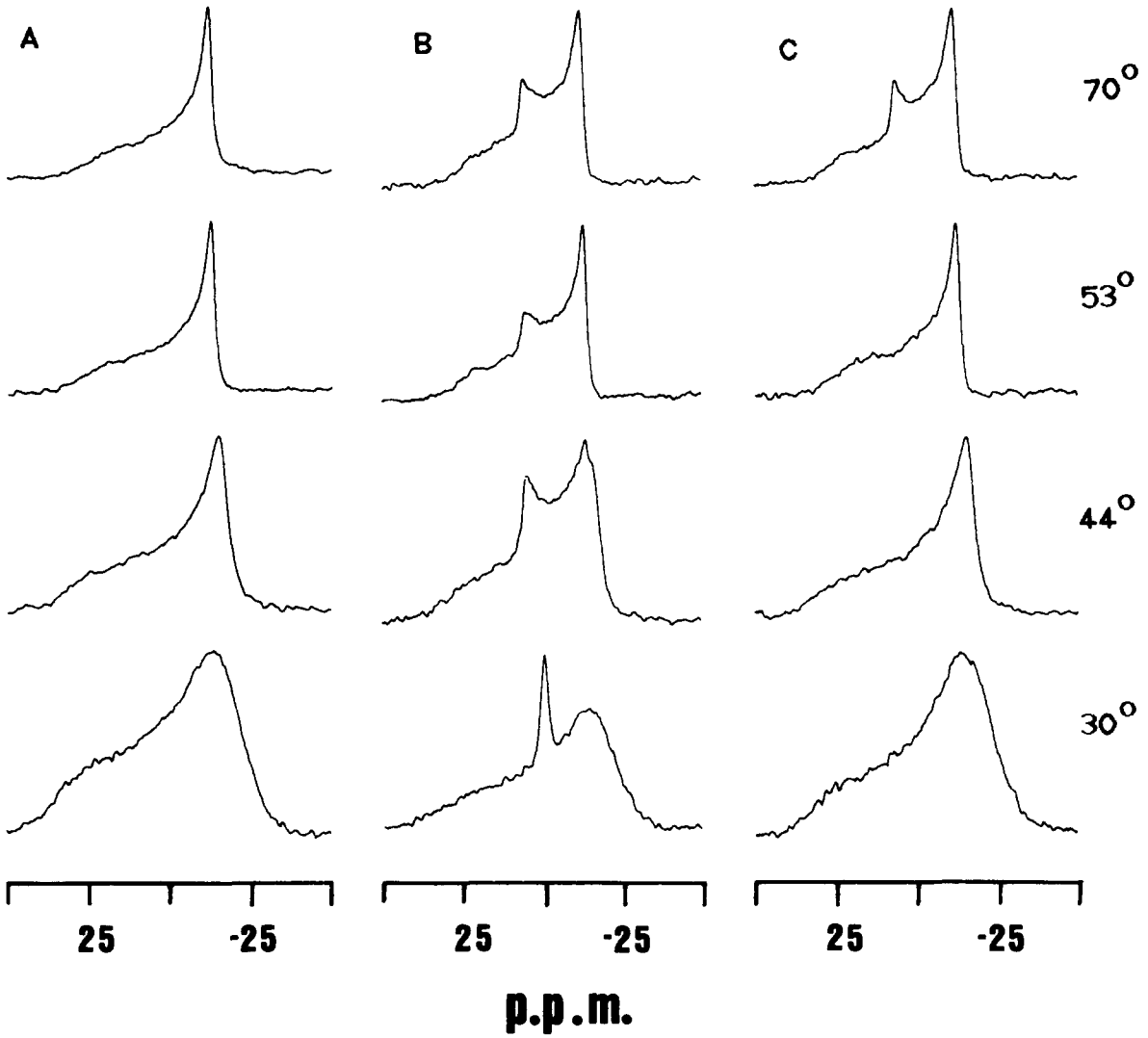

Fig. 6. ^{31}P-NMR spectra of pure DMPE (A) and DMPE containing 10 mol% α-tocopherol (B) or 10 mol% of α-tocopheryl acetate (C) at different temperatures. The spectra were normalized to the same signal height.

temperatures. We have used in this study an unsaturated PE such as DEPE, whose bilayer to H_{II} transition has been thoroughly characterized [32,33], and a saturated PE such as DMPE, which has been shown not to undergo bilayer to H_{II} transition up to 90°C [32].

We first discuss the effect of tocopherols on DEPE systems. From our calorimetric experiments it is concluded that both analogs have almost no effect on the gel to liquid-crystalline phase transition of the phospholipid, at least in the range of concentrations studied here. The effect of tocopherols on the bilayer to H_{II} phase transition is more pronounced. Incorporation of increasing amounts of both molecules results in a progressive decrease of both transition temperature and enthalpy. It can be interpreted that upon α-tocopherol or α-tocopheryl acetate incorporation, part of the DEPE molecules, most likely those interacting with tocopherols, give rise to a broad bilayer to H_{II} phase transition which is shifted to lower temperatures and which cannot be detected in the thermograms due to its width and low energy content. The remainder of the DEPE molecules still shows a slightly perturbed transition. Upon increasing the tocopherol content, the fraction of unperturbed DEPE molecules decreases, so that the enthalpy of the detectable transition decreases, and eventually only a very broad bilayer to H_{II} phase transition is present, which even starts below the temperature at which the pure phospholipid undergoes its gel to liquid-crystalline phase transition. This interpretation was confirmed by our ^{31}P-NMR experiments which showed that the bilayer to H_{II} transition of DEPE molecules is shifted to lower temperatures in the presence of tocopherols; a fraction of the DEPE molecules organize themselves in H_{II} structures even below the gel to liquid-crystalline phase transition temperature of pure DEPE. In the presence of α-tocopheryl acetate and below the gel to liquid-crystalline transition tempera-

ture, the signal arising from a fraction of the DEPE molecules is isotropic. Mixtures of bilayer and H_{II}-preferring lipid species [30] can exhibit isotropic ^{31}P-NMR signals. Such a signal is commonly observed in lipid systems intermediate between lamellar and H_{II} configuration and indicates the presence of structures in which isotropic motional averaging occurs. In our case it could indicate that in a system composed by DEPE molecules in the gel state, those molecules interacting with α-tocopheryl acetate organize themselves in an intermediate isotropic state which upon increasing temperature will adopt the H_{II} hexagonal organization.

We would like now to consider the effect of tocopherols on DMPE systems. From our calorimetric experiments it can be suggested that the effect of α-tocopherol on the gel to liquid-crystalline phase transition of DMPE is stronger and different from that of α-tocopheryl acetate. While α-tocopheryl acetate does not significantly affect this transition, the presence of α-tocopherol produces the appearance of several transition peaks. The same effect has been described for α-tocopherol on other saturated PE's [11] and it was interpreted that α-tocopherol does not give a good mixing with those PE's and lateral phase separation occurs, probably producing phases with different contents in α-tocopherol and phospholipid, so that the transition temperature will be lower as more α-tocopherol is present in each particular phase, leading to the appearance of the different peaks observed.

From our ^{31}P-NMR experiments we can gain insight into the effect of tocopherols on the macroscopic organization of DMPE. The most important finding is that, while this phospholipid organizes itself in bilayer structures at all temperatures studied, in the presence of tocopherols a fraction of DMPE molecules forms the hexagonal H_{II} phase. The presence of α-tocopheryl acetate produces a H_{II} phase but only well above the gel to liquid-crystalline phase transition. This correlates well with our calorimetric studies, as we detected a small endotherm peak at higher temperatures, this new endotherm corresponding to the bilayer to H_{II} phase transition. However, the effect of α-tocopherol is more pronounced, since it produces the hexagonal H_{II} phase at lower temperatures. It can be seen that when DMPE is in the gel state in the presence of α-tocopherol an intermediate isotropic state is found. It can be concluded that in the presence of α-tocopherol the bilayer to H_{II} phase transition of a fraction of DMPE molecules takes place at the same temperatures as those at which pure phospholipid undergoes its gel to liquid-crystalline phase transition.

The results of the present investigation demonstrate the ability of tocopherols to induce H_{II} hexagonal phase formation in DEPE and DMPE systems and that this ability is greater for α-tocopherol that for α-tocopheryl acetate, the former being also more effective in perturbing the gel to liquid-crystalline phase transition of these phospholipids.

The capacity of hydrated liquid-crystalline lipid to adopt different structures according to acyl chain or headgroup composition has been thoroughly studied. These studies have given support to the hypothesis that a generalized 'shape' property of lipids determined the phase structure adopted [34] and a shape or packing parameter, S, has been characterized [35] being $S = v/a_o l_o$ where v is the hydrophobic volume per molecule, a_o is the 'optimum' area per headgroup at the lipid/water interface, and l_o is the length of the hydrocarbon chain. Phosphatidylethanolamines have a smaller, less hydrated headgroup than phosphatidylcholines, and this together with the possibility of forming intermolecular hydrogen bonding would reduce the area per molecule in the head group region, this leading to a cone-shaped molecule, where $S > 1$, having shape properties compatible with inverted structures such as H_{II} phase. The inclusion of lipidic molecules such as tocopherols in the PE system will perturb the lipid matrix increasing the acyl chain motion which may be considered to increase the hydrophobic volume, v, and thus giving an average greater value for the shape parameter, S, and in this way facilitating the formation of the hexagonal structures by PE.

The hydroxyl group of α-tocopherol will allow this molecule to be positioned in the bilayer so that the van der Waal's interactions with the acyl chains of the phospholipid can be maximized. This would explain why α-tocopherol produced a stronger effect on PE when comparing to α-tocopheryl acetate, since α-tocopheryl acetate has its hydroxyl group blocked by the acetyl group and in doing so, it may not induce so strong a perturbation to the phospholipid structure. A stronger effect for α-tocopherol comparing to α-tocopheryl acetate on phosphatidylcholine system has also been reported [10]. It has been shown that α-tocopherol, but not α-tocopheryl acetate, may prevent the peroxidation of phospholipid in liposomes [36] and it is also well known that α-tocopherol has a biological activity different from some of its analogs.

In addition to its well-known antioxidant functions [37], vitamin E has been implicated in a series of membrane processes. It has been shown that vitamin E has fusogenic activity on erythrocytes [38] and inhibits platelet aggregation [39,40]. A correlation between erythrocyte fusion phenomena and structural variations in the bilayer organization has also been suggested [41]. It has been shown very recently that vitamin E inhibits protein kinase C activity and a role for α-tocopherol in regulating the activity of this enzyme has been suggested [42]. On the other hand, there are observations correlating liposome fusion kinetics and lipid phase behaviour [43] and also protein kinase activity correlates with the presence of non-bilayer lipid phases [44].

Taking all these observations together, it may be speculated that the influence of vitamin E on lipid polymorphism described in this paper has significance for the mechanism of action by which vitamin E exerts its activity.

Acknowledgements

We wish to thank Prof. A.V. Xavier from the Instituto Superior Tecnico, Lisboa, Portugal, for allowing us the use of his NMR facility and to Dr. H. Santos, from the same Institution, for her kind help in the NMR measurements. This work was supported by research grant PA86-0211 from CAICYT (Spain).

References

1 Burton, G.W. and Ingold, K.H. (1986) Am. Chem. Res. 19, 194–201.
2 Evans, H.M. and Bishop, K.S. (1972) Science 56, 650–653.
3 Scott, M.L. (1978) in Handbook of Lipid Research (De Luce, H.F. ed.), Vol. 2, pp. 133–197, Plenum, New York.
4 Whitaker, J., Fort, E.G., Vimokesan, S. and Dinning, J.S. (1967) Am. J. Clin. Natr. 20, 785–789.
5 Tappel, A.L. (1972) Ann. N. Y. Acad. Sci. 204, 12–28.
6 Diplock, A.T. and Lucy, J.A. (1973) FEBS Lett. 29, 205–210.
7 Witting, L. (1972) Ann. N. Y. Acad. Sci. 203, 192–198.
8 Grams, G.W. and Eskins, K. (1972) Biochemistry 11, 606–610.
9 Lai, M.Z., Düzgünes, N. and Szoka, F.C. (1985) Biochemistry 24, 1646–1653.
10 Villalaín, J., Aranda, F.J. and Gómez-Fernández, J.C. (1986) Eur. J. Biochem. 158, 141–147.
11 Ortiz, A., Aranda F.J. and Gómez-Fernández, J.C. (1987) Biochim. Biophys. Acta 898, 214–222.
12 Wassall, S.R., Thewalt J.L., Wong, L., Gorrisen, H. and Cushley, R.J. (1986) Biochemistry 25, 319–326.
13 Srivastava, S., Phadke, R.S., Govil, G. and Rao, C.N.R. (1983) 734, 353–362.
14 Severcan, F. and Cannistraro, S. (1988) Chem. Phys. Lipids. 47, 129–133.
15 Ekicl, I.H., Hughes, L., Burton, G.W., Jovall, P.A., Ingold, K.H. and Smith, I.C.P. (1988) Biochemistry 27, 1432–1440.
16 Fukuzawa, K., Ikeno, H., Tokumma, A., and Tsukatani, H. (1979) Chem. Phys. Lipids 23, 13–22.
17 Gómez-Fernández J.C., Villalaín, J., Aranda, F.J., Ortiz, A., Micol, V., Coutinho, A., Berberan-Santos, M.N. and Prieto, M.J.E. (1989) Ann. N. Y. Acad. Sci., in press.
18 Bisby, R.H. and Birch, D.J.S. (1989) Biochem. Biophys. Res. Commun. 158, 386–391.
19 Aranda, F.J., Coutinho, A., Berberan-Santos, M.N., Prieto, M.J.E. and Gómez-Fernández (1989) Biochim. Biophys. Acta 985, 26–32.
20 Cullis, P.R., Hope, M.J., de Kruijff, B., Verkleij, A.J. and Tilcock C.P.S. (1985) in Phospholipid and Celullar Regulation (Kuo, J.F., ed.), Vol. I, pp. 1–60. CRC Press, Boca Raton.
21 De Kruijff, B., Cullis, P.R., Verkleij, A.J., Hope, M.J., Van Echteld, C.J.A. and Taraschi, T.F. (1985) in The Enzymes of Biological Membranes (2nd Edn.) (Martonosi, A.N., ed.), Vol. I, pp. 131–204, Plenum Fress, New York.
22 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–471.
23 Gallay, J. and De Kruijff, B. (1984) Eur. J. Biochem. 142, 105–112.
24 Cullis, P.J. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420.
25 Epand, R.M. (1985) Chem. Phys. Lipids 36, 387–393.
26 Van Echteld, C.J.A., Van Stigt, R., De Kruijff, B., Leunnissen-Bijvelt, J., Verkleij, A.J. and De Gier, J. (1981) Biochim. Biophys. Acta 648, 287–291.
27 Killian, J.A. and De Kruijff, B. (1985) Biochemistry 24, 7881–7890.
28 Aranda, F.J. and De Kruijff, B. (1988) Biochim. Biophys. Acta 937, 195–203.
29 Seelig, J. and Gally, H.V. (1976) Biochemistry 15, 5199–5204.
30 Cullis, P.R. and De Kruijff, B. (1978) Biochim. Biophys. Acta 507, 207–218.
31 Seelig, J. (1978) Biochim. Biophys. Acta 515, 105–140.
32 Tilcock, C.P.S. and Cullis, P.R. (1982) Biochim. Biophys. Acta 684, 212–218.
33 Cullis, P.R. and De Kruijff, B. (1978) Biochim. Biophys. Acta 513, 31–42.
34 Cullis, P.R., De Kruijff, B., Hope, M.J., Verkleij, A.J., Najar, R., Farren, S.B., Tilcock, C., Madden, T.D. and Bally, M.B. (1983) in Membrane Fluidity (Alioa, R.C., ed.), Vol. 2, pp. 40–79, Academic Press, New York.
35 Israelachvili, J.N., Marcelja, S. and Horn, R.G. (1980) Q. Rev. Biophys. 13, 121–200.
36 Fukuzawa, K., Chida, H., Tokumura, A. and Tsukatami, H. (1981) Arch. Biochem. Biophys. 206, 173–180.
37 Lubin, B. and Mchlin, L.J. (1982) Ann. N.Y. Acad. Sci. 393, 1–50.
38 Ahkong, R.F., Fisher, D., Tampon, W. and Lucy, J.A. (1973) Biochem. J. 136, 147–155.
39 Steiner, M. and Anastasi, I. (1976) J. Clin. Invest. 57, 732–737.
40 Steiner, M. and Mower, R. (1982) Ann. N.Y. Acad. Sci. 393, 289–299.
41 Viti, V., Cicero, R., Callari, D., Guidoni, L., Billitteri, A. and Sichel, G. (1983) FEBS Lett. 158, 36–40.
42 Mahoney, C.W. and Azzi, A. (1988) Biochem. Biophys. Res. Commun. 154, 694–697.
43 Ellens, H., Siegel, D.P., Alford, D., Yeagle, P.L., Boni, L., Lis, L.J., Quinn, P.J. and Bentz, J. (1989) Biochemistry 28, 3692–3703.
44 De Boeck, H. and Zidovetzki, R. (1989) Biochemistry 28, 7439–7446.