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EFFECT OF α-TOCOPHEROL ON THE PHASE TRANSITION OF PHOSPHATIDYLCHOLINE AND ON WATER TRANSPORT THROUGH PHOSPHATIDYLCHOLINE LIPOSOME MEMBRANES

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Key Word Index—α-Tocopherol; phosphatidylcholine; phase transition; liposomes; water transport; temperature dependence.

Abstract—The interaction between α-tocopherol and phosphatidylcholine was studied in liposomes by differential scanning calorimetry and osmotic water transport studies. Addition of α-tocopherol to phosphatidylcholine resulted in a reduction in enthalpy at the transition temperature, a rise in osmotic water permeability of the liposomes below the phase transition temperature and disappearance of the discontinuity of osmotic water transport at the phase transition. Also the temperature dependence of osmotic water transport was reduced below the transition temperature. A comparison between cholesterol and α-tocopherol in regulation of permeability was made and the physiological relevance of tocopherol in regulation of membrane permeability is discussed.

INTRODUCTION

Tocopherol has generally been considered to prevent membrane lipid oxidation. Also it has been noted that tocopherol by its physical properties affects membrane structure. It will inhibit haemolysis of red blood cells due to excess of vitamin A [1], and will stabilize lysozome membranes of mouse liver cells [2]. In liposomes containing tocopherol, chlorophyll-a and glyco- or phospho-lipid, it fully protected linolenic acid against photo-oxidation, while chlorophyll-a degradation was only slightly reduced (glycolipid) or catalysed (phospholipid). Enhanced chlorophyll-a degradation was due to structural changes in the liposomes resulting in increased light absorption [3]. Tocopherol has a condensing effect in phospholipid monolayers [4] but it fluidizes egg phosphatidylcholine [5]. Tocopherol acetate induces a strong broadening of the phase transition temperature of dipalmitoyl phosphatidylcholine [6]. Membrane permeability of dipalmitoyl phosphatidylcholine liposomes for Pr^{3+} is increased [5] by tocopherol acetate, the transition temperature for glucose leakage is decreased as well as the glucose leakage at the optimal temperature [7]. Tocopherol acetate is miscible with dipalmitoyl phosphatidylcholine up to 40 mol % [6].

In this study the interaction between tocopherol and dipalmitoyl phosphatidylcholine in liposomes will be described further. The effect of tocopherol on the phase transition temperature of phosphatidylcholine has been repeated and the change in enthalpy at the transition temperature has been determined. In addition, the osmotic water permeability of liposomes containing dipalmitoyl phosphatidylcholine and varying amounts of α-tocopherol has been determined below, at, and above the phase transition temperature. In addition, the temperature dependence of water transport has been determined. Based on these experiments, the interactions between dipalmitoyl phosphatidylcholine and cholesterol [8–10] or tocopherol can be compared.

The results presented may be of physiological relevance, since it has been suggested that an elevated level of cholesterol may compensate in part for a lack of tocopherol in skeletal muscle of tocopherol deficient rabbits [11] and in plant root cell membranes [12].

RESULTS

Differential scanning calorimetry

Figure 1 shows that the peak of heat flow observed at the transition temperature decreased in height and broadened when increasing amounts of tocopherol were added to the phosphatidylcholine. At the same time the transition temperature decreased from 322 K to 309 K and 308 K at 20 and 30 mol % added tocopherol (Table 1). The change in enthalpy was reduced from 52 J/g lipid (no tocopherol added) to 33 J/g lipid (20 mol % tocopherol added). No significant further change in enthalpy was noted above 20 mol % added tocopherol.

Permeability studies

Figure 2 shows the temperature dependence of osmotic shrinkage of dipalmitoyl phosphatidylcholine liposomes upon osmotic shock. A steep rise in osmotic water permeability, measured as shrinkage, was visible around and above the transition temperature [Fig. 2(A)]. Below 312 K osmotic water transport was negligible. A gradual rise in osmotic water transport with temperature was observed in the range 295–312 K, i.e. at temperatures far below the phase transition temperature, 322 K, in

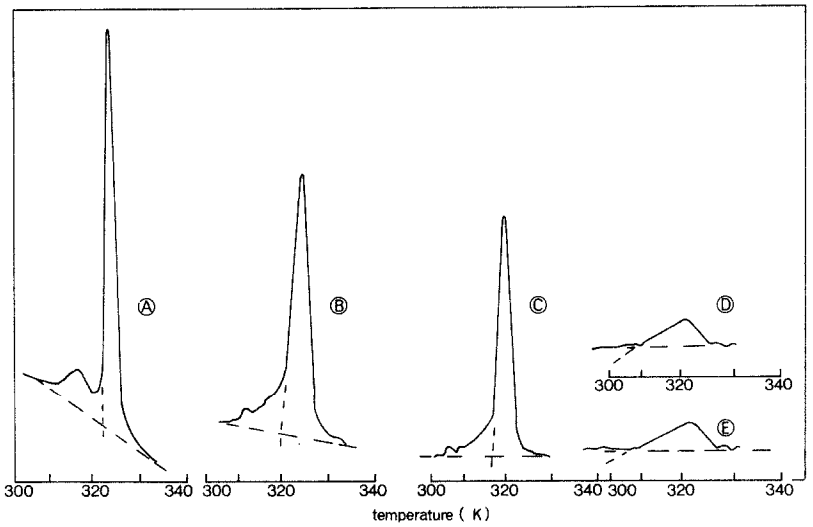

Fig. 1. Heat transition curves (differential scanning calorimetry) of dipalmitoyl phosphatidylcholine/α-tocopherol mixtures in water (1/1, by weight). A, no tocopherol added; B, 5 mol %; C, 10 mol %; D, 20 mol %; E, 30 mol % α-tocopherol.

phosphatidylcholine/tocopherol liposomes [Fig. 2(B–D)]. Above the transition temperature hardly any effect on water permeability was noted. Figure 3 shows that already a minor addition of tocopherol greatly enhanced osmotic water permeability below the transition temperature (299 K). At this temperature enhancement of water permeability was smaller above 5 mol % tocopherol and only a minor enhancement was observed above 15 mol %.

The effect of temperature on osmotic water permeability was also visualized by Arrhenius plots (Fig. 4). The strong discontinuity in the rate of shrinkage observed at the transition temperature in pure phosphatidylcholine liposomes was less pronounced when the liposomes contained 5 and 10 % tocopherol. No discontinuity was observed in liposomes containing 25 or more mol % tocopherol [Fig. 4(D)]. Activation energies were calculated from the Arrhenius plots (Fig. 5). Above the transition temperature tocopherol did not exert much influence on the activation energy (Table 2). Below the transition temperature the high activation energy for osmotic water transport observed in dipalmitoyl phosphatidylcholine decreased from 123 to 84 kJ/mol in liposomes containing 15 mol % tocopherol. For the continuous temperature curves measured for liposomes containing 25 or more mol % tocopherol an even lower value for the activation energy was observed (*ca* 75 J/mol), which was, however, higher than the value observed above the phase transition temperature in liposomes containing less tocopherol (54 kJ/mol).

For liposomes of egg phosphatidylcholine a low value for the activation energy for osmotic water transport was

Table 1. Heat of transition and phase transition temperature of dipalmitoyl phosphatidylcholine/α-tocopherol liposomes

Content of α-tocopherol (mol %)	Heat of transition (J/g lipid)	Transition temperature (K) Peak value	Onset value
0	52.1 ± 5.2	324	322
5	52.1 ± 5.2	323	320
10	48.3 ± 4.8	322	317
20	32.8 ± 4.9	321	309
30	30.2 ± 6.0	321	308

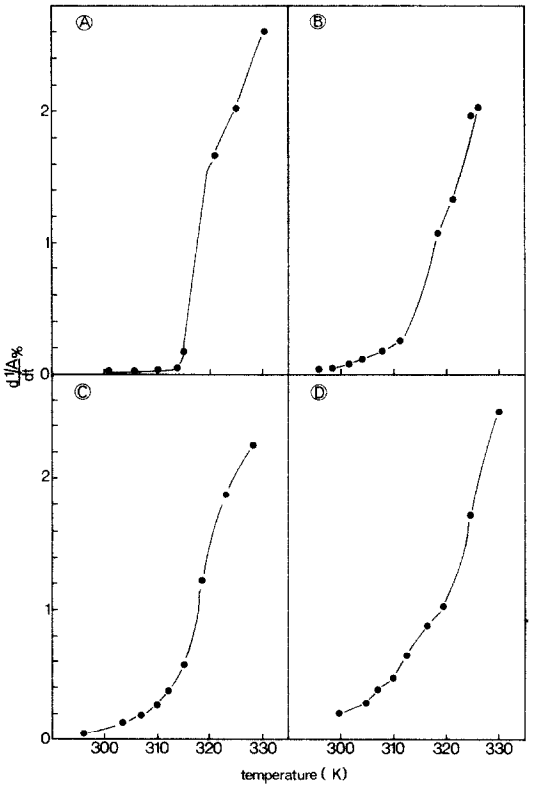

Fig. 2. Temperature dependence of osmotic shrinkage of dipalmitoyl phosphatidylcholine liposomes in hypertonic glucose. Addition of 0 (A), 5 (B), 10 (C) and 30 mol % (D) α-tocopherol.

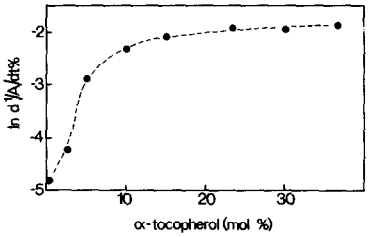

Fig. 3. The effect of α-tocopherol on osmotic shrinkage of dipalmitoyl phosphatidylcholine liposomes below the phase transition temperature (299 K).

observed, which was increased by tocopherol (25 mol %) to the same value observed in dipalmitoyl phosphatidylcholine/tocopherol liposomes (75/25 by percentage).

DISCUSSION

Addition of α-tocopherol to dipalmitoyl phosphatidylcholine reduced the heat of transition and lowered the transition temperature in a similar way as cholesterol [8]. It seems that dipalmitoyl phosphatidylcholine may accommodate up to 40 mol % tocopherol or tocopherol acetate [5], and up to 50 mol % cholesterol [8]. The fact that the heat of transition did not differ significantly between preparations containing 20 and 30 mol % tocopherol and that it still remained rather high (contrary to cholesterol) indicates that at these concentrations the miscibility of tocopherol with dipalmitoyl phosphatidylcholine is rather limited.

Differences between dipalmitoyl phosphatidylcholine liposomes containing tocopherol or cholesterol, as far as osmotic water permeability is concerned, can be summarized as follows. Cholesterol and tocopherol both reduce the discontinuity of the temperature dependence of

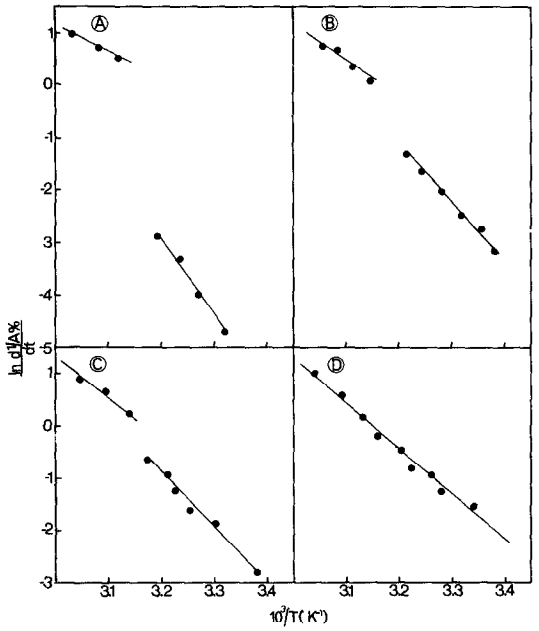

Fig. 4. Arrhenius plots of osmotic shrinkage of dipalmitoyl phosphatidylcholine liposomes, containing 0 (A), 5 (B), 10 (C) and 30 (D) mol % α-tocopherol.

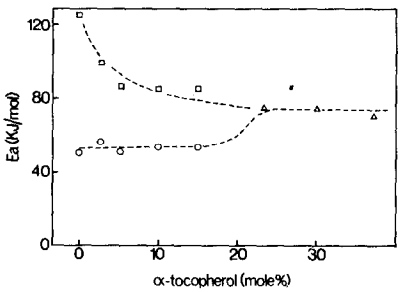

Fig. 5. The effect of α-tocopherol on the activation energy of osmotic shrinkage of dipalmitoyl phosphatidylcholine liposomes. ○---○, above the phase transition temperature; □---□, below the phase transition temperature; △---△, continuous temperature curve.

osmotic shrinkage of dipalmitoyl phosphatidylcholine liposomes, but tocopherol (5 mol %) is more effective in this respect than cholesterol (20 mol %). Continuous temperature curves for osmotic water transport are observed at smaller amounts of added tocopherol than is the case with cholesterol. Below the transition temperature a stronger decrease in activation energy for osmotic water transport is observed by addition of tocopherol than by cholesterol [10]. Above the transition temperature the activation energy is slightly increased by tocopherol while cholesterol addition results in a steep rise of activation energy at *ca* 15 mol % [10]. Cholesterol and tocopherol both increase the temperature dependence of osmotic water transport of egg phosphatidylcholine liposomes (above the phase transition temperature). Increasing ratios of cholesterol/phosphatidylcholine and tocopherol/phosphatidylcholine are accompanied by a decrease in osmotic water permeability above the phase transition temperature and osmotic water transport is stimulated by cholesterol and tocopherol below the transition temperature [13, 14].

Table 2. Activation energies for osmostic water transport through phosphatidylcholine/tocopherol liposomes above and below the phase transition temperature

Content of α-tocopherol (mol %)	Activation energy (kJ/mol)		
	Discontinuous temperature curves		Continuous temp. curves
	Below T_c	Above T_c	
Dipalmitoyl phosphatidylcholine			
0	123 ± 12	47 ± 3	
2.5	98 ± 2	56 ± 3	
5	86 ± 3	50 ± 9	
10	85 ± 2	54 ± 7	
15	84 ± 2	54 ± 4	
25			78 ± 1
30			76 ± 2
37.5			71 ± 3
Egg phosphatidylcholine			
0		45 ± 3	
25		71 ± 4	

It is suggested that the isoprenoid side chain of α-tocopherol with its protruding methyl side chains is responsible for the effects observed in dipalmitoyl phosphatidylcholine/tocopherol liposomes. Contrary to the condensing effect of cholesterol, the isoprenoid chain of tocopherol may produce a large number of 'kinks' in the hydrophobic area of the liposomes [15] thus increasing the free volume for water and facilitating osmotic water transport. Thus glucose permeability is also stimulated below the phase transition temperature [7]. It will be interesting to see if the described difference between cholesterol and tocopherol, as far as interaction with phosphatidylcholine is concerned, also occurs in biomembranes, e.g. in vitamin E-deficient tissues with an elevated cholesterol level [11] or in plant root cell membranes [12]. It is evident that similar studies on membrane compounds like plant sterols, quinones and other lipophilic vitamins may contribute towards an understanding of the effects of these compounds on permeability of plant cell membranes.

EXPERIMENTAL

Lipids. Cholesterol and dipalmitoyl phosphatidylcholine (both 99% pure) were obtained from Sigma (Saint Louis, U.S.A.); 2-*ambo*-α-tocopherol (99% pure) was obtained from Merck (Darmstadt, BRG). Egg phosphatidylcholine was obtained from egg yolk [16]. The purity of phosphatidylcholine was checked by TLC (Si gel, $CHCl_3$–MeOH–H_2O, 13:5:1). Egg phosphatidic acid was prepared from egg phosphatidylcholine by phospholipase D degradation.

Calorimetry. Measurements were made with lipids and lipid mixtures dispersed in H_2O (1:1, by wt). Thermal analysis was done with a Perkin–Elmer DSC-2B instrument. Samples were assayed at a heating rate of 8°/min.

Permeability measurements. Multilayered liposomes containing 4 mol % egg phosphatidic acid were prepared as described by De Gier *et al.* [13]. Liposomes prepared in 10 mM Tris–HCl (pH 7.5) were osmotically shocked by injecting a sample of liposomes (0.7 μmol lipid/ml) in a 0.1 M glucose soln of the same temp., using an Aminco Stopped Flow Apparatus. Changes in turbidity were determined with a spectrophotometer (Aminco DASAR Control type) at 450 nm. From the recording tracings the shrinkage velocity, $[d(1/A)/dt]\,[100/A_0]$ at $t = 0$ was calcd as described by Blok *et al.* [9,10]. Activation energies were calculated by the least square method from Arrhenius plots, presenting ln $[d\ 1/A/dt]\%$ against $1/T$, T being the absolute temperature (K).

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