Thermotropic Interaction of Vitamin E with Dimyristoyl and Dipalmitoyl Phosphatidylcholine Liposomes

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Summary The effect of vitamin E on the thermal behavior of two saturated phosphatidylcholines was investigated by differential scanning calorimetry. For dimyristoyl and dipalmitoyl phosphatidylcholines, the addition of vitamin E at concentrations of 1, 5 and 10 mol% vitamin E, resulted in a lowering of the phase transition temperature and a broadening of the temperature range of the phase transition indicating an increase in the fluidity of the phospholipids. Taken together with other physical studies on the effect of vitamin E on (unsaturated) phospholipids, these results indicate that vitamin E could influence the physical properties of membrane phospholipids in addition to its known antioxidant role. The likelihood of this interaction would be enhanced if vitamin E was not randomly distributed in biological membranes but rather was located in domains where its local concentration, relative to phospholipids, was elevated.

Key Words vitamin E (α -tocopherol), differential scanning calorimetry, phase transitions, phospholipids

The lipid soluble compound vitamin E (α -tocopherol) is considered to play a vital role as a lipid antioxidant and free radical scavenger protecting cell membranes from the damaging effects of various oxygen metabolites (1). However, it has been suggested that in addition to its antioxidant role, vitamin E may play a structural role in biological membranes and influence the physical state of biological membranes by altering lipid-lipid and/or lipid-protein interactions (2, 3). Evidence of a structural role for vitamin E comes in part from the effect of vitamin E on the fluidity characteristics of human platelet membranes (4) and the aggregation properties of platelets (5). Additional evidence comes from the effect of vitamin E on the growth and transport properties of cultured cells (6, 7), the surface behavior of lipid monolayers and the transport properties of liposomes (2). ^{31}P NMR and fluorescence anisotropy changes are also observed in erythrocyte ghost membranes as a result of the addition of vitamin E and indeed other lipophilic vitamins (8).

The physical interaction of lipid-soluble compounds with membranes and

isolated lipids, has been investigated by studying the phase transition behavior of such systems. By using the non-invasive technique of differential scanning calorimetry (DSC), measurement can be made of the transition temperature, the transition width and the enthalpy of the transition. This information can indicate how the presence of a drug or lipid-soluble compound may alter the physico-chemical properties of lipids and thus allow insight into the biochemical and physiological implications of such interactions (9-12).

This study examines the effect of vitamin E on the phase transition behavior of aqueous dispersions of dimyristoyl and dipalmitoyl phosphatidylcholine using differential scanning calorimetry. The fatty acid chains of these two phospholipids display clearly defined, highly cooperative order-disorder phase transitions at temperatures of 23 and 41°C, respectively (13).

EXPERIMENTAL

To stock solutions of dimyristoyl L- α -phosphatidylcholine (DMPC) or dipalmitoyl L- α -phosphatidylcholine (DPPC) (Sigma) in chloroform was added vitamin E (Sigma) in ethanol to give final concentrations of 0, 1, 5 and 10 mol% of vitamin E in DMPC or DPPC. After concentrating the various mixtures under a stream of N₂, 5 to 10 mg of each mixture was added to 75 μ l stainless-steel pans (Perkin-Elmer, Norwalk, CT, USA) and dried of solvents by vacuum desiccation for 24 h. Forty μ l of 50 mM Tris/2 mM EDTA/15% (v/v) ethylene glycol (pH 7.2) (liposome buffer), was then added to each pan. Pans were immediately sealed and allowed to hydrate for 24 h. Prior to thermal examination, pans were vortexed (but not sonicated), for 5 min at a temperature just above the aqueous phase transition temperature of the particular phospholipid. All manipulations prior to sealing of the pans were performed under dim light and the (distilled) solvents and liposome buffer were extensively bubbled with N₂.

Thermal scans were performed using a Perkin-Elmer Differential Scanning Calorimeter (Model DSC-2B) and analyzed using a Perkin Elmer Thermal Analysis Data Station as previously described (14). Water and an indium standard (Perkin-Elmer) were used for temperature and enthalpy calibration. Scans were made at 5°C per min against an empty sealed reference pan. All samples were scanned at least three times in both the cooling and heating modes. The onset temperature has been defined as that temperature at which a significant departure from the baseline is first evident.

RESULTS

The effects of increasing vitamin E concentration on the thermotropic behavior of DMPC and DPPC are shown in Figs. 1 and 2 respectively. Both the exothermic (cooling) and endothermic (heating) transitions were significantly broadened with increasing vitamin E concentration (Fig. 3). At higher con-

Fig. 1. Representative differential scanning calorimetric (DSC) scans of aqueous dispersions of dimyristoyl phosphatidylcholine (DMPC) with varying amounts of vitamin E. The scan rate in either the cooling or heating modes was 5°C/min. Scans have been normalized to 5 mg dry weight of total lipid for graphic presentation. The vertical bar represents 0.2 mcal/s.

Fig. 2. Representative DSC scans of aqueous dispersions of dipalmitoyl phosphatidylcholine (DPPC) with varying amounts of vitamin E. Scans and data presentation are as described in Fig. 1.

centrations, particularly 10 mol%, vitamin E caused a decrease in the enthalpy of the transition for both phospholipids (Table 1). Also apparent was a reduction in the transition temperature (T_m) upon increasing the vitamin E level to 5 mol%.

Fig. 3. Width of the phase transition (in centigrade degrees) at half the transition height for aqueous dispersions of DMPC and DPPC containing various amounts of vitamin E examined in either the cooling (●) or heating (▲) modes.

Table 1. Effect of vitamin E on the heat and temperature of transitions for DMPC and DPPC.

The phase transition temperature (T_m) defined as the onset temperature for both cooling and heating runs, is shown in °C. The enthalpy (ΔH) is shown in kcal mol⁻¹.

Mol% Vit. E	DMPC				DPPC			
	Cool		Heat		Cool		Heat	
	T_m	ΔH	T_m	ΔH	T_m	ΔH	T_m	ΔH
0	22.7	-6.9	25.7	6.3	40.0	-9.0	43.4	8.4
1	20.7	-6.3	22.4	6.3	40.5	-9.1	41.7	8.4
5	20.7	-6.3	19.5	5.8	39.4	-8.1	38.2	8.1
10	23.1	-5.4	19.2	5.7	39.9	-7.0	37.3	7.4

slightly elevated T_m at 10 mol% vitamin E was probably due to the large extent of transition broadening evident at this concentration of vitamin E. The pretransition for both phospholipids was not observed at concentrations of vitamin E of 1 mol% or greater (Fig. 1). Aqueous dispersions of vitamin E alone exhibited no detectable phase transition when examined under the conditions outlined in this study (data not shown) which is in accord with the spin label studies of Srivastava *et al.* (15).

DISCUSSION

Vitamin E at the lowest tested concentration of 1 mol% had a significant effect on the thermotropic behavior of both saturated phosphatidylcholines. This effect consisted of a loss of the pretransition peak, broadening of the main peak and in most instances, reduction in the phase transition temperature. These effects were

accentuated on higher vitamin E concentrations. However, significant effects on the enthalpy of the main transition were not evident until at least 5 mol% vitamin E was present in the liposomes (except for DMPC in the cooling mode).

In general our results bare some similarity to those described by Massey *et al.* (16), but some important differences were apparent. Firstly, we have extended our study to include the interaction of vitamin E with dipalmitoylphosphatidylcholine rather than just myristic acid containing phospholipids as was done in the Massey *et al.* study. For most biological membranes, palmitic acid-containing phospholipids (along with those containing stearic acid) greatly exceed those containing myristic acid (17, 18). Thus our study has greater relevance to the situation which normally exists in biological membrane lipids with regard to the saturated fatty acid components. Secondly, as our DSC data were obtained with both heating and cooling scans, it would indicate that at a concentration as low as 1 mol%, vitamin E affects the pretransition peak and the transition width in aqueous dispersions of both phospholipids when they are either in the fluidus (liquid-crystalline) or solidus (gel) states. Previous DSC studies indicate that at least mitochondrial membrane lipids are in the liquid-crystalline state at body temperature (14) and thus the studies we report here would have relevance to the normal physical situation existing for membrane lipids *in vivo*.

The lowering of the phase transition temperature would suggest that vitamin E increases the fluidity of the hydrocarbon chain region of DMPC and DPPC liposomes, whilst the increase in the width of the transition would probably indicate that a reduction has occurred in the size of the cooperative unit of lipids participating in the acyl chain phase transition (11). Similar effects have been observed with general anaesthetics, some local anaesthetics and some aliphatic alcohols of 5 to 10 carbons (11). Abolition of the pretransition peak has previously been observed when compounds that reduce the phase transition temperature are added to phospholipid dispersions (11).

Using thermal techniques similar to those employed in this study, Chapman (19) concluded that those compounds that penetrate the interior of the lipid bilayer and disrupt the chain packing, result in a lowering of the heat or enthalpy of the lipid phase transition. Those compounds that remain near the surface of the bilayer and interact electrostatically with the polar headgroups of lipids, primarily affect the transition temperature. Thus the studies reported here would suggest that at low concentrations of vitamin E (< 1 mol%), the interaction may initially be near the surface of the bilayer being promoted by hydrogen bonding, as judged by the decrease in the phase transition temperature. It has been postulated that binding of vitamin E to phospholipids can occur by the formation of a hydrogen bond with either of the four oxygens of the phosphate group or with the oxygen atoms in the glycerol moiety (15). Increasing the concentration to 5 mol% or greater leads to a lowering of both the enthalpy and the transition temperature (primarily by increased peak broadening). This may indicate that vitamin E also penetrates into the interior of the lipid bilayer causing disruption to the chain

packing and reduction in the cooperative unit.

Although this study only investigated the interaction of vitamin E with saturated phosphatidylcholines, there is clear evidence that vitamin E perturbs these phospholipids at a concentration of 1 mol%. However, the net result of vitamin E/phospholipid interactions may differ depending on both the fatty acids associated with membrane phospholipids and the concentration of vitamin E in the membrane system under study. Indeed, other studies have shown that the penetration of vitamin E into phospholipid monolayers is greatly facilitated by the presence of relatively small quantities of unsaturated phospholipids (2). Thus for the polyunsaturated phospholipids present in most biological membranes, specific physico-chemical interactions may occur between the phytanyl side chain of vitamin E and the fatty acyl chains of membrane lipids, particularly those derived from arachidonic acid (2). Using stearic acid spin label probes, Ohki *et al.* (20) demonstrated that vitamin E increases the order parameter in a concentration-dependent manner in a fraction of vitamin E above 0.2 molar in rat liver phosphatidylcholine liposomes whereas the order parameter of egg yolk phosphatidylcholine was unaffected. The different effect of vitamin E was attributed to the difference in the arachidonic acid content of the two systems, with rat liver phosphatidylcholine having the far higher content of arachidonic acid (20).

With regard to the content of vitamin E in biological membranes, very few if any literature values (expressed as mol% of membrane phospholipid) are available to allow one to postulate on *in vivo* interactions between vitamin E and membrane phospholipids. We have recently analyzed human platelet vitamin E content using HPLC and obtained a value of approximately 10 µg/g wet weight of platelets (McIntosh and McMurchie, unpublished data). This value was doubled in individuals actively supplementing their diet with vitamin E. Based on a percent recovery of phospholipids from platelets of 0.6% of the wet weight, the vitamin E concentration was calculated at about 0.3 mol% (phospholipid) increasing to 0.6 mol% in vitamin E supplemented individuals.

Using literature values for the vitamin E content of various tissues and membranes, which have been expressed in a variety of ways, we have estimated vitamin E content of tissues in the form of mol% (of phospholipid) from the particular study indicated. For example, we calculate 0.13 mol% for rat liver mitochondria from the data of Taylor *et al.* (21); 0.4 mol% for rat brain cortex (22); 0.05 mol%, rat liver mitochondria (23); 0.1 mol%, human platelets (24); 0.15 mol%, human platelets (25); 1.0 mol%, rat liver microsomes (20); 0.6 mol%, rat platelets (26); 0.33 mol%, rat lung (27). Together with our data this gives a range of 0.1 to 1.0 mol%.

From the present study it would appear that although vitamin E has the capacity to physically interact with membrane phospholipids at a concentration of 1 mol%, vitamin E is probably not present in biomembranes at the gross level in amounts sufficient to allow such an interaction to occur. However, the possibility exists that vitamin E is present in certain domains within the membrane where its

local concentration could be sufficient to perturb such membrane lipid structure.

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