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Differential scanning calorimetric study of the effect of vitamin D_3 on the thermotropic phase behavior of lipids model systems

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Abstract

Differential scanning calorimetry has been used to investigate the effects of vitamin D_3 on the physical properties of model membranes including pure phosphatidylcholines (PC's) of chain lengths from 14 to 18, pure dipalmitoylphosphatidylethanolamine (DPPE), and various mixtures of these lipids. The results demonstrate that the interactions of vitamin D_3 with PC's are dependent on acyl chain lengths. It was found that vitamin D_3 reduces the transition temperatures of PC's and PE's, broadening the transition and reducing the enthalpy, eventually abolishing the transition. The interaction of vitamin D_3 with PC varies with chain length; the transition of DMPC is abolished by only 15 mol% vitamin D_3 whereas 45 mol% vitamin D_3 is required to abolish the transition of DSPC. These variations in vitamin D_3 lipid interactions are further explored in various mixtures. In the mixture studies it is shown that vitamin D_3 affects the mixing properties of the lipids in the mixtures. The results suggest that the presence of vitamin D_3 in lipids can affect the lateral phase distributions of lipids, and thereby have important effects on membrane function.

Keywords: Vitamin D_3; Model membrane; Phospholipid; DSC

1. Introduction

The vitamin D_3 endocrine system is well characterized as a major participant in the regulation of calcium metabolism in humans [1]. The parent compound, vitamin D_3, is photosynthesized in the epidermis or is ingested. Vitamin D_3 is bioactivated through two sequential hydroxylation steps; first in the liver to form 25-hydroxyvitamin D_3 and then in the kidney to form the biologically active metabolite 1,25$(OH)_2$ vitamin D_3 through the enzyme 25$(OH)D_3$-1α-hydroxylase.

Like other steroid hormones, vitamin D_3 regulates gene expression in a variety of target cells [2] and also regulates cell proliferation and mediates differentiation of several cell types, including hematopoietic (activated lymphocytes), epidermal cells [3] and different kinds of melanoma cells, in particular prostatic epithelial and stromal cells [4,5]. Intracellular vitamin D_3 receptors have been identified now in a wide variety of tissues not involved in Ca metabolism and also in cultured cells [2,6]. The presence of a protein receptor in a cell confers on that cell the capability of becoming a 'target cell', i.e. producing a new biological response as a consequence of the presence of the hormone. These examples show an important additional aspect of vitamin D_3 action, which is its involvement in growth and differentiation processes of different tissues.

Vitamin D_3 and its metabolites may regulate membrane enzyme activity such as phospholipase A_2 and calcium transport across the membrane, at least in part by inducing a specific interaction in membrane phospholipid [7]. Changes in the phospholipid deacylation/reacylation cycle can significantly alter membrane parameters such as permeability, transport, and fluidity. This is the case for intestinal epithelium, where 1,25$(OH)_2$ vitamin D_3 results in changes in the saturation of membrane phospholipid

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DMPC, L-α-phosphatidylcholine dimyristoyl; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DSC, differential scanning calorimetry.

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and, ultimately, membrane fluidity [7,8]. Recently it was reported that vitamin D_3 and its derivatives have an antioxidant ability, which may be of importance in protecting the membrane of normal cells against free radical-induced oxidative damage [9].

All of these examples suggest the importance of the presence of vitamin D_3 in membranes, including the physical effect on membrane lipids. In the present paper differential scanning calorimetry (DSC) has been used to study the effects of vitamin D_3 on the thermodynamic properties of several phosphatidylcholines (PC) and phosphatidylethanolamines (DPPE), and their mixtures.

2. Materials and methods

2.1. Chemicals

Vitamin D_3 was purchased from Sigma, St. Louis, MO. All phospholipids were obtained from Avanti Polar Lipids, Pelham, AL.

2.2. Liposomes preparation

The liposomes used for DSC measurements were prepared as follows. Appropriate aliquots of lipid and vitamin D_3 solutions in ethanol were mixed in order to obtain homogeneous mixtures at various molar ratios. The mol% vitamin D_3 in the liposomes varied between 2 mol% and 45 mol%. The solvent was removed by drying using N_2 gas and then samples were kept in a vacuum 2–3 h. Double distilled water was added to the resulting film. The exact concentration of lipid was determined by the method of Bartlett [10]. Multilamellar liposomes were formed by mixing using a vortex mixer and keeping the samples at a temperature above the phase transition temperature of the higher melting lipid until a homogeneous and uniform suspension was obtained [11].

2.3. High-sensitivity differential scanning calorimetry

DSC measurements were performed using the MC 2 scanning calorimeter from MicroCal, Amherst, MA. The calorimeter is interfaced with an IBM compatible computer and the software used is Origin software provided by MicroCal. The temperature decrease for cooling scans was provided by a Haake FC 3 refrigerated bath which was controlled by the computer. For DSC experiments, the stock liposome sample was held at room temperature until it was loaded into the calorimeter. The lipid concentration for DSC studies were between 0.8 and 4.0 mg/ml lipid . Both heating and cooling scans were employed for the studies of the main transition, and the scan rates were 20 C°/h for both heating and cooling scans. The data were analyzed using ORIGIN software from Microcal, Amherst, MA. [12].

3. Results

3.1. The effect of vitamin D_3 on saturated phosphatidylcholines

The DSC scans in Fig. 1a-c demonstrate the effect of the incorporation of increasing quantities of vitamin D_3 on the main phase transition of the DMPC, DPPC, and DSPC. These data show that as little as 2 mol% vitamin D_3 introduces a significant perturbation in these phospholipids. In addition, the pretransition is completely abol-

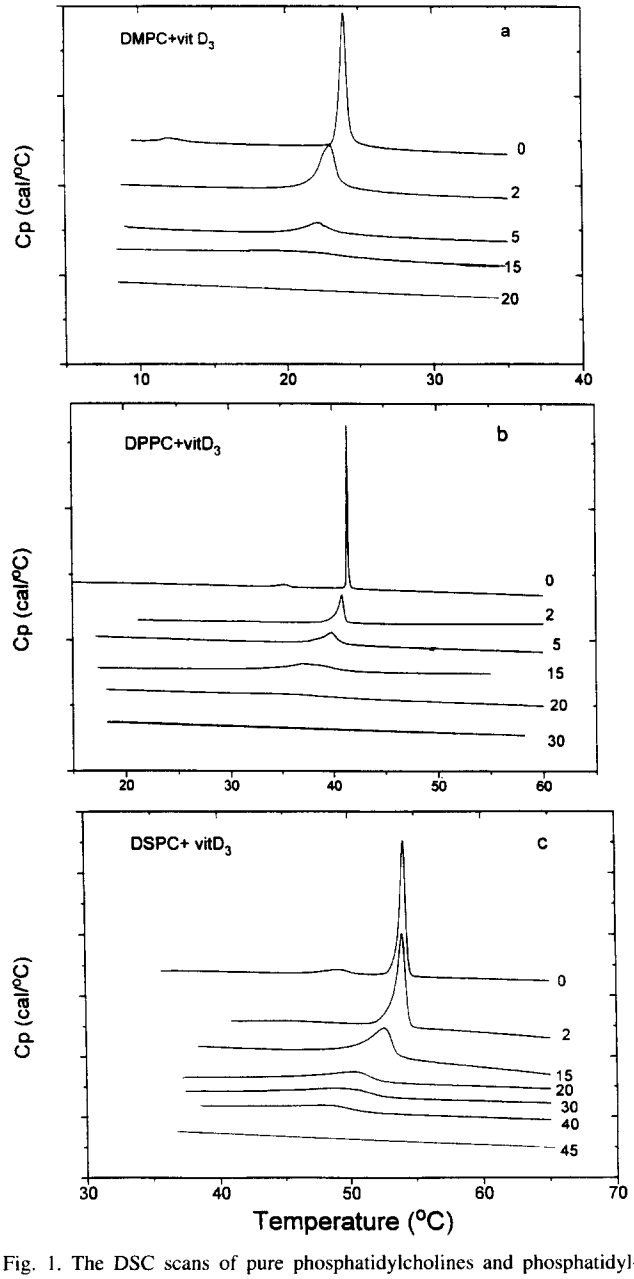

Fig. 1. The DSC scans of pure phosphatidylcholines and phosphatidylcholine/vitamin D_3 systems. Molar % contents of vitamin D_3 are indicated on the curves. (a) DMPC-containing samples; (b) DPPC-containing samples; (c) DSPC-containing samples.

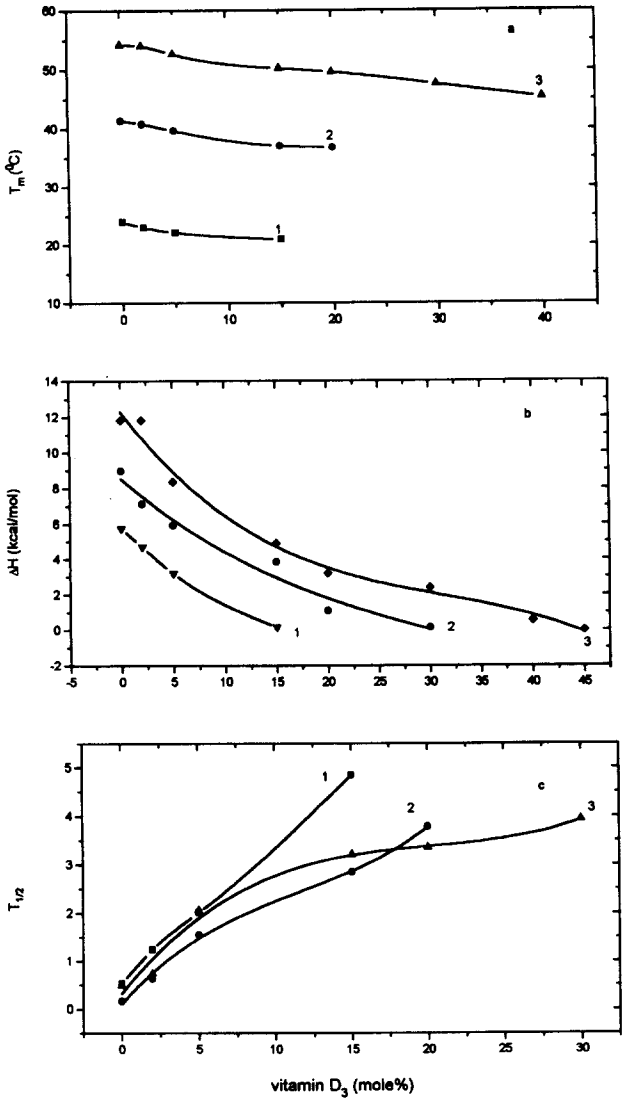

Fig. 2. Effect of vitamin D_3 on the thermodynamic properties of DMPC (trace 1), DPPC (trace 2), and DSPC (trace 3) as a function of vitamin D_3 content. (a) Temperature of the main phase transition (T_m). (b) Total enthalpy (ΔH). (c) Half-height width ($T_{1/2}$).

ished at these concentrations of vitamin D_3 for each of these lipids. Both heating and cooling scans were performed for each study; the cooling scans are not shown because the scans were completely reversible.

The effect of vitamin D_3 on the main phase transitions varies with lipid chain length. The main transitions were broadened and shifted to lower temperatures for each PC, and eventually abolished. Fig. 2a shows that in the presence of vitamin D_3, the temperature of the phase transition decreases for the three phospholipids as a function of the vitamin D_3 content. The slopes of the plots are similar, with the maximum temperature depression of nearly 9 degrees for DSPC. The temperature shift indicates that vitamin D_3 partitions preferentially into the liquid crystal phase.

Fig. 2b shows the effect of vitamin D_3 on the enthalpy of the transition, showing that the enthalpy decreases in a non linear way for each of the PC's as a function of vitamin D_3 content until it is abolished. The amount of vitamin D_3 which abolished the main transition varied with lipid chain length. For DMPC the main transition totally disappeared at 20 mol%, for DPPC at 30 mol%, and for DSPC at 45 mol% vitamin D_3. The phase transition is abolished when the presence of vitamin D_3 is sufficient to prevent the formation of a discrete gel phase. These results suggest that vitamin D_3 disrupts the gel phase of the short chain lipid more than that of the longer chain lipids. This may be related to the relative chain lengths of each of the lipids compared to the vitamin D_3.

Fig. 2c shows the increases in the width of the transition as a function of vitamin D_3 concentration. It appears that the transition widths for DMPC and DPPC are more affected than that of DSPC at the high concentrations of vitamin D_3. This is consistent with the finding that more vitamin D_3 is required to abolish the phase transition of DSPC than the short chain lipids.

Fig. 3 is an expansion of Fig. 1c, showing the asymmetry of the transition of DSPC at vitamin D_3 concentrations from 5 to 20 mol%. These can be deconvoluted into a sharp and broad component. DPPC also showed this behavior; however the transitions of DMPC remained symmetric for all concentrations. This asymmetry of the tem-

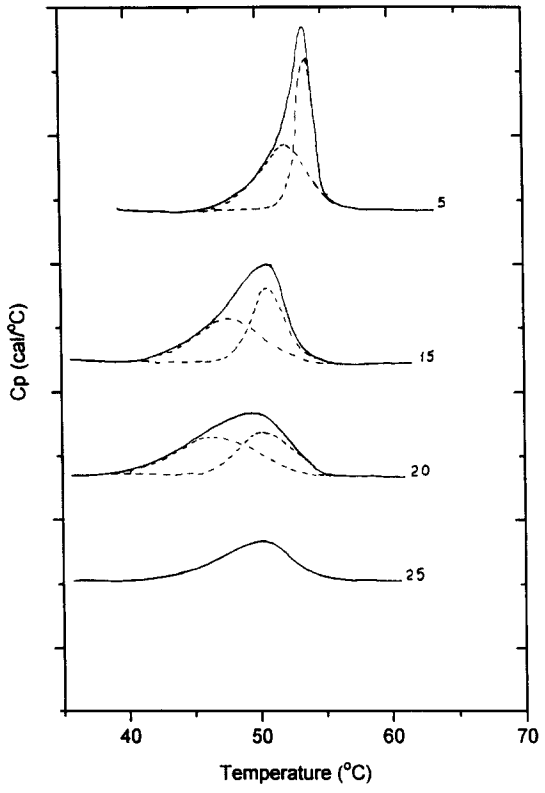

Fig. 3. Sample deconvolution of the DSC endotherms for four concentrations of vitamin D_3 in DSPC liposomes.

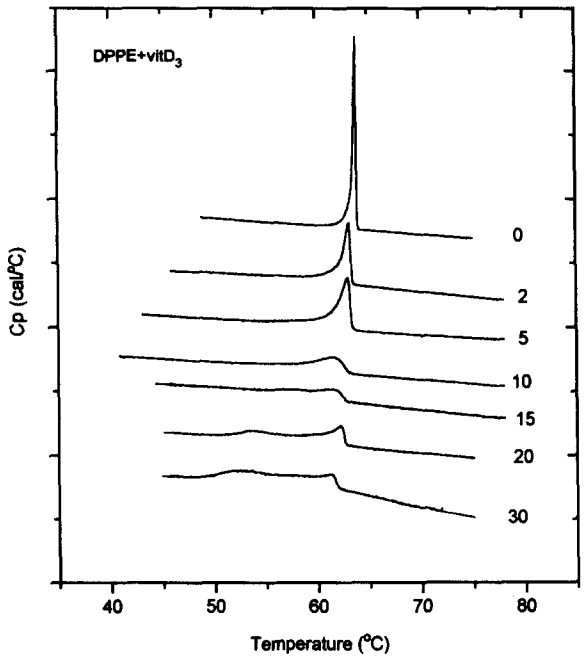

Fig. 4. The DSC scans for pure DPPE containing different amounts of vitamin D_3. Molar % contents in vitamin D_3 are indicated on the curves.

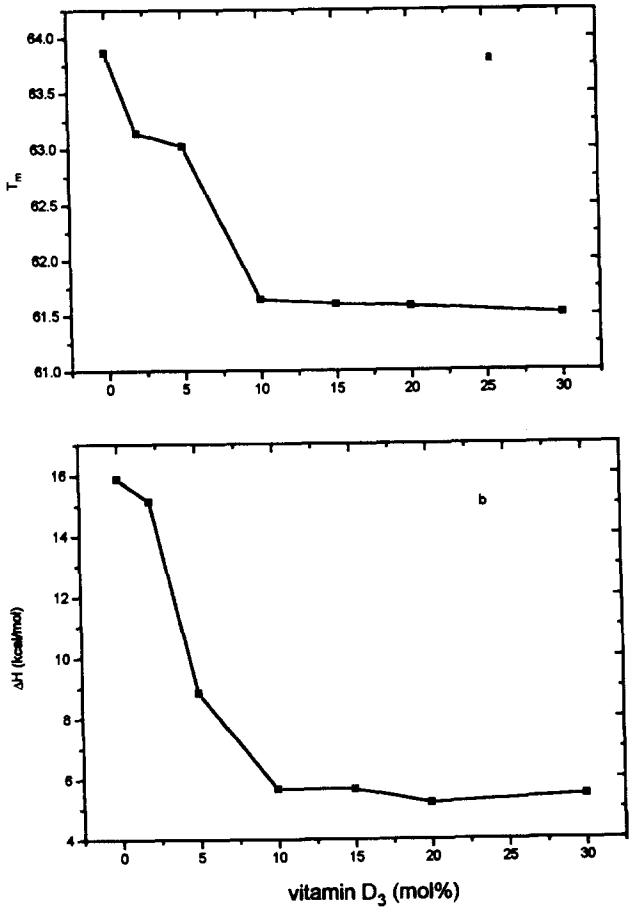

Fig. 5. Effects of vitamin D_3 on the thermodynamic properties of DPPE. (a) Changes in the temperature of the main phase transition (T_m), and (b) total enthalpy (ΔH) for the mixture of vitamin D_3 with DPPE.

perature shift suggests that the preferential partitioning of vitamin D_3 into the liquid crystal phase over the gel phase is enhanced by longer acyl chains, leading to a gel phase which is more depleted of vitamin D_3 near the liquidus boundary for the longer chain lipids. This is consistent with the observation that more vitamin D_3 is required to abolish the phase transition of the long chain lipid compared to the short chain lipid. This may be due to the hydrophobic mismatch between the effective length of the vitamin D_3 molecule compared to that of the PC hydrocarbon chains [13,14].

3.2. The effect of vitamin D_3 on phosphatidylethanolamine

In the DSC studies of PE's, the first scan was often different from the second scan; however there were no further changes for subsequent scans. It is well known that aqueous suspensions of saturated phosphatidylethanolamine can form a crystalline phase which can reform only slowly and that the transition behavior depends on the thermal history of the sample [15,16]. In the present study we used DSC data from the second scan. All scans were reversible.

Fig. 4 shows DSC data for pure DPPE and for DPPE with increasing concentrations of vitamin D_3. Fig. 5a,b show the T_m and the enthalpy for the transition as a function of vitamin D_3 content. At low concentrations of vitamin D_3 (2–10 mol%) the temperature of the phase transition was slightly shifted to lower temperature (Fig.

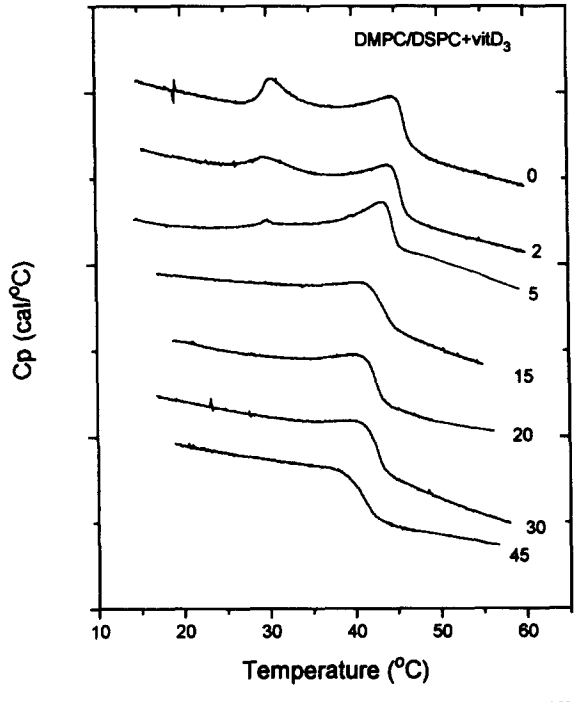

Fig. 6. The DSC scans for DMPC/DSPC mixtures containing different amounts of vitamin D_3. Molar % contents in vitamin D_3 are indicated on the curves. All the samples contain equimolar amounts of both phospholipids.

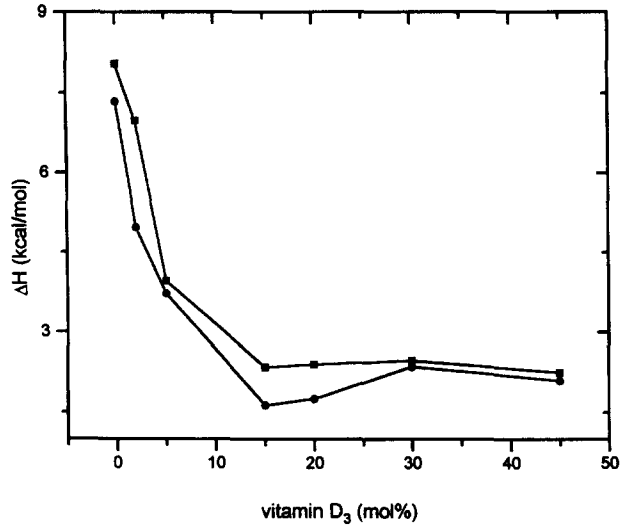

Fig. 7. Changes in total enthalphy (ΔH) for 50:50 DMPC/DSPC mixture as a function of vitamin D_3 content. ■, heating scan; ●, cooling scan.

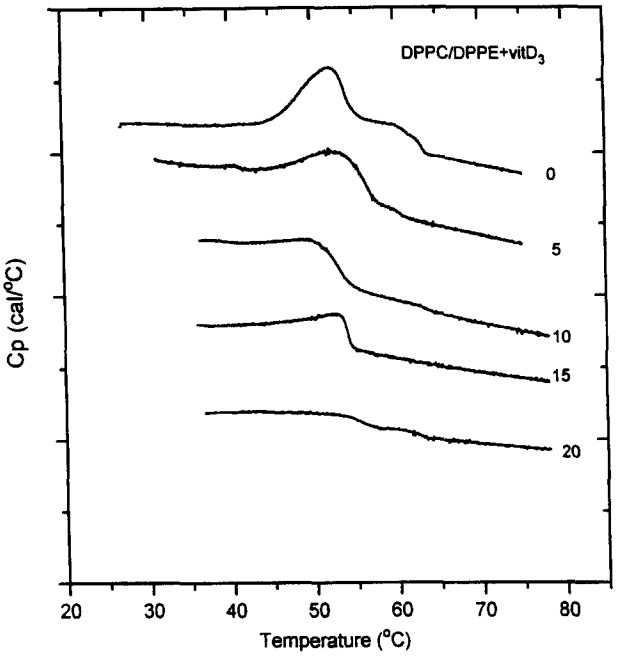

Fig. 8. The DSC scans for 50:50 DPPC/DPPE mixture as a function of vitamin D_3 content. Molar % contents in vitamin D_3 are indicated on the curves.

5a) and the enthalpy decreased (Fig. 5b). This indicates that in PE, as in PC, vitamin D_3 partitions preferentially into the liquid crystal phase. Beginning with 10 mol% of

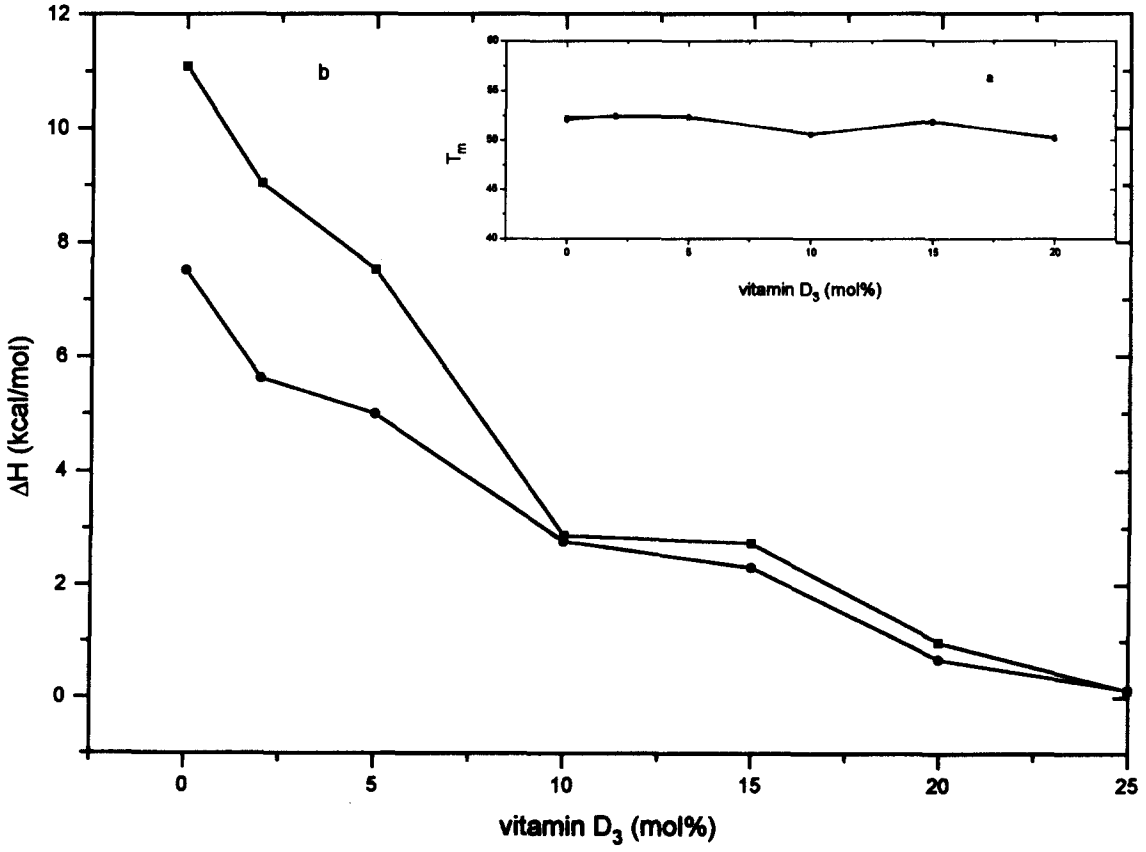

Fig. 9. Changes in the temperature of the main phase transition (T_m) and total enthalpy (ΔH) for a 50:50 DPPC/DPPE mixture as a function of vitamin D_3 content. ■, heating scan; ●, cooling scan.

vitamin D_3 there were no further significant changes in the temperature of the phase transition and the enthalpy decreased very slowly. This is in contrast to the PC's where the decrease in T_m continued throughout the concentration range studied, and it suggests that vitamin D_3 is not fully miscible with the gel phase of DPPE. As indicated in Fig. 4, at high concentrations of vitamin D_3 (20–30 mol%) a second peak appeared in DPPE with T_m approx. 53°C, suggesting the separation of a vitamin D_3 rich domain which has a low enthalpy transition.

3.3. Effect of vitamin D_3 on the mixtures of phosphatidylcholines

The effect of vitamin D_3 upon the thermotropic behavior of mixtures of various phosphatidylcholines can give us information about whether vitamin D_3 is better accommodated in one or the other of the molecular species of phosphatidylcholines present. We have investigated the mixture of DMPC and DSPC which shows monotectic behavior [17,18]. Fig. 6 shows the effect of increasing concentrations of vitamin D_3 on a 50 mol% mixture of DMPC and DSPC. It shows that vitamin D_3 at low concentrations first affects the lower temperature transition. The higher temperature transition is relatively unaffected up to 5 mol%, while the lower temperature transition has disappeared at 15 mol% of vitamin D_3. At this point the higher temperature transition is broadened, with gradually decreasing enthalpy and T_m. The higher temperature transition was still present at 45 mol% vitamin D_3 whereas this concentration completely abolished the phase transition in the pure DSPC, as shown in Fig. 1c. This suggests that the preferential partitioning of the vitamin D_3 into the liquid crystal phase is greater when the liquid crystal phase contains a significant amount of the short chain DMPC. The greater persistence of the high temperature peak in the mixture compared to the pure DSPC system is the result of the greater depletion of the vitamin D_3 in the gel phase. Fig. 7 shows the total enthalpy of the transition as a function of vitamin D_3, which decreases rapidly up to 15 mol% vitamin D_3, and then decreases very gradually. The initial rapid decrease reflects the disappearance of the lower temperature transition.

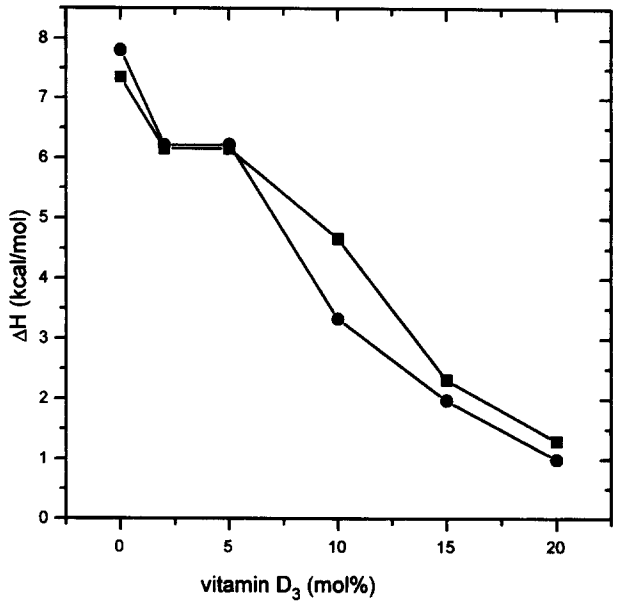

Fig. 11. Changes in total enthalpy (ΔH) for the 50:50 DMPC/DPPE mixture as a function of vitamin D_3 content. ■, heating scan; ●, cooling scan.

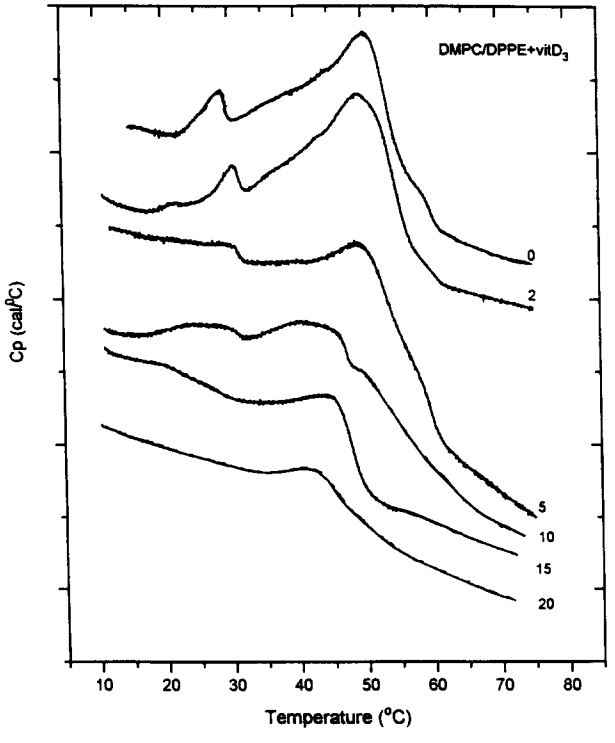

Fig. 10. The DSC scans of 50:50 DMPC/DPPE mixtures as a function of vitamin D_3 content. Molar % contents in vitamin D_3 are indicated on the curves.

3.4. Effect of vitamin D_3 on mixtures of phospatidylcholine and phosphatidylethanolamine

Phosphatidylcholines and phosphatidylethanolamines constitute the major glycerophospholipid species found in biological membranes. The balance of these phospholipids in natural membranes is very important for membrane function. In the present studies the effects of vitamin D_3 on the two equimolar PE/PC mixtures, DPPE/DPPC, which is partially miscible, and DPPE/DMPC, which shows immiscibility the gel phase, were investigated.

Fig. 8 shows scans of equimolar mixtures of DPPE/DPPC with increasing amounts of vitamin D_3. In the absence of vitamin D_3 the mixture gives a wide transition with a main peak at 52.3°C and a shoulder at 60.0°C. When increasing concentrations of vitamin D_3

were incorporated the higher temperature shoulder merged with the low temperature peak. This suggests that vitamin D_3 enhances the mixing of DPPC and DPPE. As shown in Fig. 9a,b the temperature of the transition did not change but ΔH was decreased as more vitamin D_3 was included in the mixtures. The transition is totally suppressed at 25 mol% of vitamin D_3, whereas in the pure DPPE the transition persists up to greater than 30 mol% vitamin D_3. This suggests that vitamin D_3 is more readily incorporated into the mixture, disrupting the gel phase, than into the pure lipid.

The second PC/PE mixture studied was the equimolar mixture of DMPC and DPPE which shows solid phase immiscibility as seen in Fig. 10 [19]. Incorporation of 2 mol% vitamin D_3 produced a shift to higher temperature of the lower temperature transition (to $T_m = 31.6°C$) in contrast to the behavior found for pure DMPC, where vitamin D_3 produced a decrease in the temperature of the phase transition. At the same time the high temperature transition was broadened at this concentration of vitamin D_3 and its temperature maximum decreased. This suggests that as little as 2 mol% vitamin D_3 enhances the miscibility of DMPC and DPPE in the gel phase. At 5 and 10 mol% of vitamin D_3 both transitions were broadened and the temperature of the higher temperature transition was further decreased. At 15 mol% vitamin D_3, the lower temperature peak disappeared. As seen in Fig. 10 20 mol% vitamin D_3 was not sufficient to abolish the higher melting transition, in contrast to the DPPC/DPPE mixture. Fig. 11 shows the progressive decrease of the total enthalpy as a function of vitamin D_3 content. These data indicate that the presence of vitamin D_3 in the both DMPC/DPPE and DPPC/DPPE mixtures enhances the gel phase miscibilities of the lipids.

4. Discussion

In the present investigation we have studied the effects of vitamin D_3 on the phase behavior of several phospholipids. The results we have obtained are interesting and suggest that vitamin D_3 may have important effects on membranes through its effects on the membrane lipids. We have shown that the effects of vitamin D_3 vary considerably with acyl chain length and/or lipid headgroup, and that vitamin D_3 can alter the phase separations in mixtures. These findings suggest that the biological effects of vitamin D_3 can be affected by and can affect lipid compositions and lateral phase separations in biological membranes, which can in turn affect many membrane functions.

Our studies of the effects of vitamin D_3 on the phase behavior of PC's showed significant variation as a function of acyl chain length of the lipid. The melting temperatures and enthalpies for each lipid are reduced as a function of vitamin D_3 content, indicating a preference for the liquid-crystal phase [20]. However, the amount of vitamin D_3 required to eliminate the main phase transition varied as a function of chain length from 15 mol% vitamin D_3 for DMPC to 45 mol% for DSPC. The phase transitions of DMPC and DPPC remained symmetrical as vitamin D_3 was increased; however, DSPC showed the induction of some phase separation by the addition of vitamin D_3 This effect may be due to the mismatch in length of the vitamin D_3 and the lipid acyl chains so that the vitamin D_3 is less miscible in the longer chain lipid, and less disruptive of the gel phase. Acyl chain length dependencies of cholesterol-PC interactions have also been observed and attributed to acyl chain mismatch [13,14,21–23].

The effect of vitamin D_3 on the phase behavior of DPPE was qualitatively different from its effects on PC's. The initial scans were irreproducible, as has been reported for PE's [15,16,24,25], but the second scan results were stable over several subsequent scans. It was shown that the presence of vitamin D_3 resulted in asymmetrical scans at all concentrations. At 30 mol% vitamin D_3 the main transition is still visible, at only slightly decreased transition temperature, and an additional peak suggesting phase separation has appeared at lower temperature. These results suggest that vitamin D_3 is more immiscible in PE than in PC. This may be due to the more stable interfacial region of the PE's due to its lower hydration compared to the PC's. An early study of cholesterol interaction with PE's did not show this type of phase separation, however the relatively low sensitivity of that study may have prevented detection of such a peak [26].

In order to assess the relative mixing properties of vitamin D_3 with the various lipids, its effects in several binary lipid mixtures were examined. It was found that vitamin D_3 preferentially interacts with the lower-melting lipid in the mixture of two PC's, suggesting that vitamin D_3 prefers the liquid-crystalline phase over the gel phase. Similar results were observed for the incorporation of cholesterol with mixtures of PC's which showed phase separation. In these systems the cholesterol also preferentially interacted with the lower melting component [13,27].

In the mixtures of PC with PE, however, the results were qualitatively different. In the case of the generally immiscible mixture DMPC/DPPE, the vitamin D_3 shifted the temperature of the main transition to higher temperature, indicating that vitamin D_3 enhanced the miscibility of the two lipids in the mixture [28,29]. Similar results were observed for the more miscible mixture DPPC/DPPE. Incorporation of vitamin D_3 into the mixture led to increased miscibility of these lipids also. This is in contrast to some early studies of mixtures of PE and PC with cholesterol, in which cholesterol showed preferences for PC component and did not enhance the mixing behavior. The results reported in the present studies suggests that both lipid chain length and head group affect the mixing behavior of vitamin D_3 with lipids. They also show that

the presence of vitamin D_3 can enhance the miscibilities of lipids in mixtures. These effects may be due to the relative mismatches of the molecular length of vitamin D_3 compared to the lipid chain length, as well as disruption of the interfacial regions of gel phase lipids.

Other studies in the literature have shown that vitamin D_3 metabolites and other steroid hormones have a direct effect on the membrane fluidity [7,30], showing that $1,25(OH)_2$ vitamin D_3 and $24,25(OH)_2$ vitamin D_3, estradiol and others stimulated membrane fluidity in epithelial cells in vivo and in isolated membranes in vitro. These changes in fluidity are important because the plasma membrane is the locus of early cell response to external influence. In addition, membrane fluidity plays a role in regulation of membrane-bound enzyme activity due to the requirement of such enzymes for a specific lipid microenvironment [31]. It has also been reported that α-tocopherol [32], ubiquinone-3 [33], and vitamin K-1 [34], also induce a broadening and decease of the phase transition of DPPC but this influence was not chain length-dependent.

The results reported here demonstrate that the interactions of vitamin D_3 with individual lipids vary depending on both the headgroup and the chain length of the particular lipid. It has also been shown previously that the presence of vitamin D_3 in DPPC has an influence on the interactions of calcium ions with this lipid [35]. These observations suggest that the distribution of vitamin D_3 in biological membranes is influenced by the lipid composition, as well as the ionic milieu. The differences in the interaction of vitamin D_3 with PC's and PE's suggest also that vitamin D_3 may have an asymmetrical distribution in biological membranes. These studies taken together suggest that vitamin D_3 may be one among the many substances which can contribute to the regulation of biological membrane functions.

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