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A CALORIMETRIC STUDY OF THE THERMOTROPIC BEHAVIOUR OF 1,2-DIPENTADECYLMETHYLIDENE PHOSPHOLIPIDS

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Summary

Differential scanning calorimetry was used to study the thermotropic behaviour of 1,2-dipentadecylmethylidene phospholipids with various head groups. The structural variation in the glycerol backbone region leads to a strong restriction of conformational freedom for the first two methylene segments of the chains, so that dipentadecylmethylidene phospholipids show lower transition temperatures, lower enthalpies and lower cooperativity of the transition from the gel to the liquid crystalline phase. The extreme chemical stability of these lipids in the alkaline pH region enables investigations of phosphatidylethanolamine and phosphatidic acid dispersions at high pH values. Both phospholipids show a decrease in the transition temperature and in the transition enthalpy as they become singly and doubly charged, respectively. A complex behaviour of the transition enthalpy of doubly charged 1,2-dipentadecylmethylidene phosphatidic acid was observed when the NaCl concentration of the dispersion was increased.

Introduction

The characteristics of the gel-to-liquid crystalline phase transition of phospholipid bilayers depend on parameters such as the structure of the fatty acid

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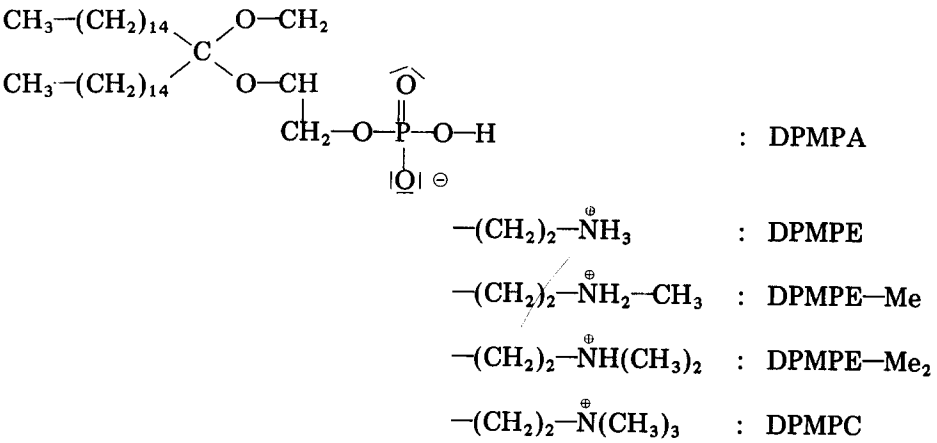
Abbreviations: DPMPA, 1,2-dipentadecylmethylideneglycero-3-phosphoric acid; DPMPE, 1,2-dipentadecylmethylideneglycero-3-phosphoethanolamine; DPMPE-Me, 1,2-dipentadecylmethylideneglycero-3-phospho-*N*-methylethanolamine; DPMPE-Me_2, 1,2-dipentadecylmethylideneglycero-3-phospho-*N*,*N*-dimethylethanolamine; DPMPC, 1,2-dipentadecylmethylideneglycero-3-phosphocholine.



Scheme I

chains, the position of the acyl residue within the glycerol molecule and the nature of the polar head group [1—6]. Structural modification of the apolar region may include alteration in chain length, unsaturation and branching. The polar region may vary in structure or charge. All phospholipids with ester linkages of the hydrocarbon chains seem to adopt a similar molecular conformation in which the chain at the 2-position of the glycerol backbone is bent, so that the ends of the hydrocarbon chains are not in register [7,8]. This particular conformation may be maintained in phospholipids with ether linkages of the chains because these lipids show very similar phase transition characteristics, though their transition temperatures are usually several degrees higher [9—11].

In continuation of our work on the influence of structure and charge on the physical properties of phospholipid/water systems, it was the aim of this study to construct phospholipid molecules with a severe restriction of conformational freedom in the glycerol backbone region. Such molecules are, for instance, 1,2-dipentadecylmethylidene phospholipids. The ketal structure incorporates the 1,2-oxygens of the glycerol molecule into a five-membered ring system. Thus, the molecular conformation of this specific region is fixed and the characteristic bending of ester and ether chains at the 2-position of glycerophospholipids is prevented. These structural peculiarities should be also reflected in physical properties such as the phase transition temperature and enthalpy which are discussed in this paper. In addition, the high degree of chemical stability of these lipids in the pH range from 7 to 12 allows the study of the effect of charge alteration on phosphatidylethanolamine and phosphatidic acid systems.

Materials and Methods

DPMPC, DPMPE-Me, DPMPE-Me_2, DPMPE and DPMPA were synthesized as described elsewhere [12]. The purity of the starting dipentadecyl ketone was more than 98%.

The calorimetric measurements were made as described before, using the adiabatic differential scanning calorimeter developed by Grubert [11,13]. The lipid concentrations used were in the range from 1 to 2 mg/ml water. The dispersions were prepared by sonicating the dry lipid in approx. 20 ml of double-distilled water for 2 or 3 min above the respective phase transition temperature of the lipid. This procedure produces essentially large multilayer liposomes. The dispersion was then transferred to the calorimetric vessel and made up to 25 ml with double-distilled water. The pH of the dispersions was adjusted with dilute NaOH and controlled before and after each calorimetric run.

Heats of transition were determined by measuring the area under the excess heat capacity vs. temperature curves by paper weighing.

NaOH and NaCl were p.a. grade (Merck, Darmstadt, F.R.G.).

Results

The calorimetric scans of the five different dipentadecylmethylidene phospholipids investigated are shown in Fig. 1. The transition behaviour is quite similar to that known for the diester and diether phospholipids. At pH 8, the transition temperature, T_m, of phosphatidic acid is highest next to phosphatidylethanolamine. Methylation of phosphatidylethanolamine leads to an almost linear decrease in T_m with the result that DPMPC has the lowest transition temperature (Fig. 2a).

DPMPE-Me_2 has a peculiar transition behaviour, since two maxima in the excess heat capacity curve were observed (Fig. 1). This may indicate that this lipid is partly deprotonated at pH 8. In fact, DPMPE-Me_2 has the lowest pK value of the three phosphatidylethanolamines of different degrees of methylation [12].

The alteration of the transition enthalpy, ΔH, is more complex (Fig. 2b). Phosphatidylethanolamine has the highest ΔH value, 5.6 kcal/mol at pH 8. The introduction of the first methyl group results in a more than 25% decrease

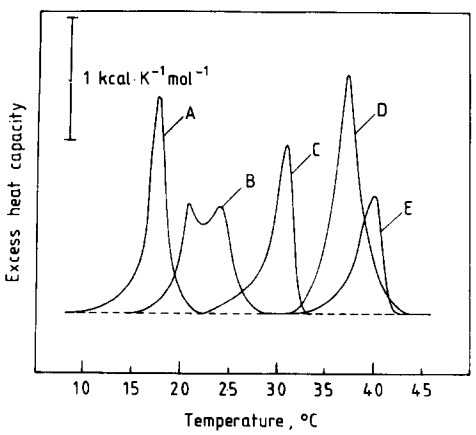


Fig. 1. Calorimetric scans of 1,2-dipentadecylmethylidene phospholipids in aqueous dispersions at pH 8.0: (A) DPMPC, (B) DPMPE-Me, (C) DPMPE-Me2, (D) DPMPE, (E) DPMPA.

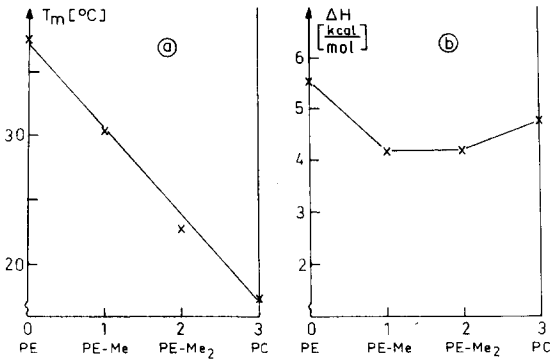


Fig. 2. (a) Transition temperature T_m and (b) transition enthalpy ΔH of 1,2-dipentadecylmethylidene phospholipids as a function of methylation of the ethanolamine head group. PC, phosphatidylcholine; PE, phosphatidylethanolamine.

in the ΔH value, from 5.6 to 4.2 kcal/mol for DPMPE-Me. The incorporation of a second and a third methyl group is then of minor influence on the values of ΔH which are quite similar for all of the three methylated phosphatidylethanolamines including choline.

When the van't Hoff transition enthalpies are evaluated from the integrated calorigrams, the size of the cooperative units can be calculated from the ratio $\Delta H_{\text{van't Hoff}} : \Delta H_{\text{cal}}$ [14]. The size of the cooperative unit ranges from 40 to 60 molecules for the dipentadecylmethylidene phospholipids. Thus, the cooperativity of the transition for these lipids seems to be lower than for the diester phospholipids [15]. Since the size of the cooperative unit depends strongly on the presence of small amounts of impurities [16], a good comparison would need dipentadecylmethylidene phospholipids of purities higher than 98%. For a transition in a two-dimensional hexagonal lattice, the relationship:

$$n = \frac{\Delta H_{\text{van 't Hoff}}}{\Delta H_{\text{cal}}} = \frac{1}{(3\sqrt{\sigma} - 2)}$$

holds, where σ is the cooperativity parameter [17,18]. The cooperative interaction energies, $RT\ln\sigma$, calculated from these cooperative units deviate only by approx. 3% from the critical value calculated from $\sigma = 2/3$, which gives a first-order transition. Thus, only small changes in the cooperative interaction energies can lead to large changes in the size of the cooperative unit.

Due to the chemical stability of dipentadecylmethylidene phospholipids at alkaline pH values, the pH dependence of the transition of DPMPE and DPMPA was determined in the pH range from 8 to 13 and compared to the known behavior of ester and ether lipids [10,11]. The transition temperature of DPMPE decreases drastically with deprotonation of the ammonium group which results in the formation of one negative charge per phosphate group. In Fig. 3, the calorimetric scans for DPMPE at different alkaline pH values are shown. The difference in T_m for DPMPE between pH 8 and 13 is 19°C (Fig. 4) in comparison to 21°C for dipalmitoyl phosphatidylethanolamine (Blume, A., unpublished results) and 25°C for dihexadecyl phosphatidylethanolamine [19]. With increasing charge, the ΔH values become smaller. A value of 5.6

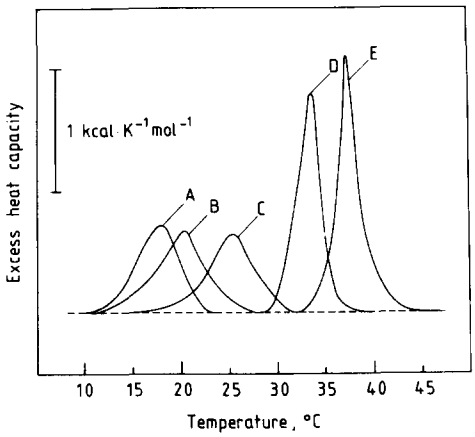


Fig. 3. Calorimetric scans of DPMPE at the following pH values: (A) pH 13.1, (B) pH 12.0, (C) pH 11.0, (D) pH 10.0, (E) pH 7.0.

kcal/mol is found for pH 8 and one of 4.0 kcal/mol for pH 13.

The transitions of DPMPE-Me and DPMPE-Me_2 are also influenced by deprotonation at alkaline pH values. However, the extent of the effect is smaller, since in the deprotonated state at pH 12 the phosphatidylethanolamines and phosphatidylcholines have almost the same phase transition temperatures, T_t (16—18°C), and ΔH values, 3.6 kcal/mol for DPMPE-Me and 3.6 kcal/mol for DPMPE-Me_2.

As expected from earlier studies on the transition behaviour of diester and diether phosphatidic acids, the phase transition temperature of DPMPA is shifted to lower temperatures with increasing pH. Calorimetric scans of DPMPA at different pH values are shown in Fig. 5. A variation in the pH value from 8 to 12 results in a decrease in T_m of 20°C (Fig. 6a). The transition enthalpy again shows lower values when the phosphatidic acid is double charged in agreement with previous observations (Fig. 6b).

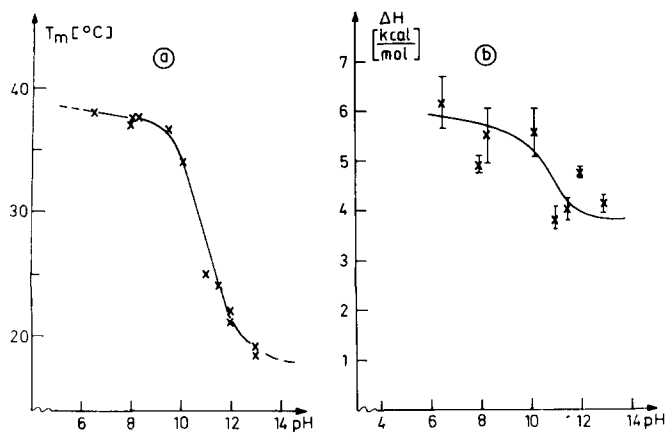


Fig. 4. (a) Transition temperature T_m and (b) transition enthalpy ΔH of DPMPE as a function of pH.

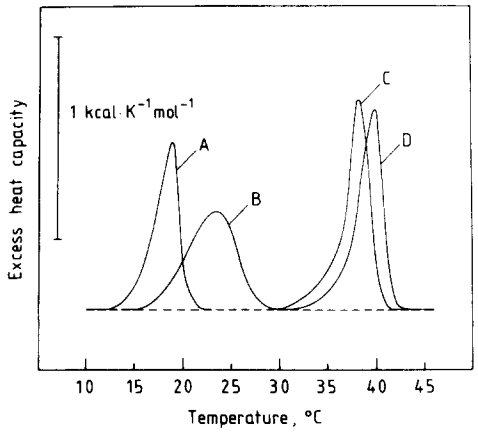


Fig. 5. Calorimetric scans of DPMPA at the following pH values: (A) pH 12.2, (B) pH 9.3, (C) pH 7.5, (D) pH 6.5. The dispersions contained 0.04 M NaCl.

Finally, the influence of increasing salt concentrations (Na^+) at pH 12 on the transition characteristics of DPMPA was studied. A slight increase in the T_m value linear with the square root of the NaCl concentration was observed, $1°C/(mol/l)^{1/2}$. A larger dependence of T_m on the salt concentration was described earlier for methylphosphatidic acid [21]. As shown in Fig. 7. a dramatic change in the transition enthalpy was observed with increasing NaCl concentration; for a concentration of 0.2 mol/l, ΔH is 2 kcal/mol higher than in water. Further addition of salt then leads to a slight decrease in ΔH.

Discussion

It is well known that the length and the degree of unsaturation and branching, as well as the nature of the polar head group of phospholipids, determine the characteristics of the bilayer phase transition. On the other hand, changes

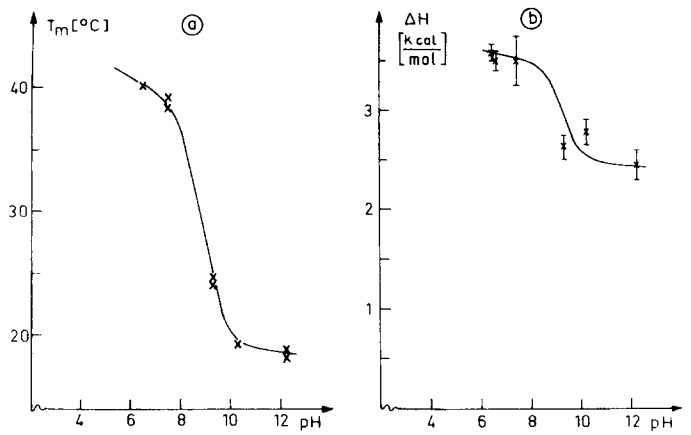


Fig. 6. (a) Transition temperature T_m and (b) transition enthalpy ΔH of DPMPA as a function of pH in dispersions containing 0.04 M NaCl.

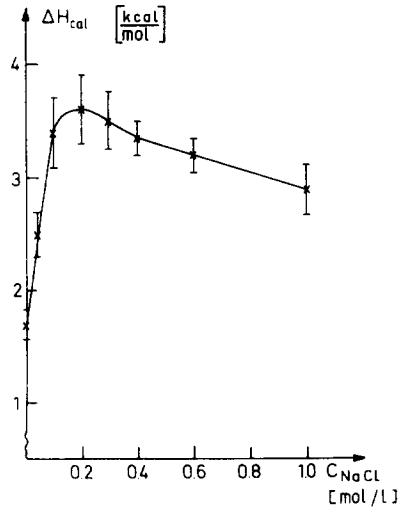


Fig. 7. Transition enthalpy ΔH of DPMPA at pH 12.0 as a function of NaCl concentration.

at the glycerol backbone, especially a different kind of linkage of the hydrocarbon chains to the glycerol molecule should also influence the transition behaviour. The hydrocarbon chains of most of the phospholipids are linked to the glycerol molecule via ester bonds. The two acyl chains are inequivalent due to a conformation in which the chain at the 2-position of the glycerol molecule first extends parallel to the bilayer and then proceeds downward so that the chain ends are not in register. This conformation was first determined by Hitchcock et al. [7] from X-ray investigations of dilauroyl phosphatidylethanolamine crystals and obviously is maintained in the bilayer structures of all phospholipids with ester linkages [8]. In some biological membranes the hydrocarbon chains are linked to the glycerol molecule via ether bonds [22]. Ether phospholipids show only a slightly different behav-

TABLE I

A COMPARISON OF THE ΔH VALUES AND OF THE TRANSITION TEMPERATURES, T_m, OF DIPENTADECYLMETHYLIDENE PHOSPHOLIPIDS WITH ETHER AND ESTER PHOSPHOLIPIDS

(a) ΔH values (in Kcal/mol) at pH 7, (b) T_m values (in °C) at pH 7. HH, 1,2-dihexadecyl-*sn*-glycero-3-; PP, 1,2-dipalmitoyl-*sn*-glycero-3-; MM, 1,2-dimyristoyl-*sn*-glycero-3- and DPM, 1,2-dipentadecylmethylidene-*rac*-glycero-3-; PA, -phosphoric acid; PE, -phosphoethanolamine; PE-Me, -phospho-*N*-methylethanolamine; PE-Me_2, -phospho-*N*,*N*-dimethylethanolamine; PC, -phosphocholine.

		PA	PE	PE-Me	PE-Me_2	PC
(a)	HH	5.4	7.5	—	—	8.5
	PP	7.8	8.2	—	—	8.1
	MM	5.6	6.4	—	—	6.0
	DPM	3.6	5.6	4.2	4.2	4.8
(b)	HH	71.0	68.0	—	—	42.9
	PP	65.0	64.3	—	—	41.0
	MM	50.5	49.6	—	—	23.0
	DPM	38.5	37.5	31.0	24.0	17.9

iour than the corresponding ester lipids. Their transition temperatures are usually somewhat higher (2—5°C) and their transition enthalpies are different [9,11,19,20]. The reason for this may lie in a different degree of hydration of the glycerol backbone and a closer packing of the hydrocarbon chains. However, the exact molecular conformation of both chains in ether phospholipids is not known and discussion about the inequivalence of the 1- and 2-chains in the case of ether analogues is speculative.

The chemical structure of the phospholipids discussed here differs fundamentally from those with ester or ether bonds. The oxygen atoms of the glycerol backbone are part of a five-membered ring system which introduces a severe restriction on mobility in this region of the molecule and also alters the extent of intramolecular interactions which depend on structural and steric effects. Therefore, drastic changes of the transition behaviour can be expected, since the peculiar arrangement of dipentadecylmethylidene phospholipids will prevent a conformational array where the 2-chain is bent. No reduction of the effective chain length of either chain can occur as described for ester phospholipids.

The chain length of the dipentadecylmethylidene phospholipids is 16 carbon atoms, including the ring carbon which belongs to both chains. Schematically, it compares with dipalmitoyl phospholipids or dihexadecyl phospholipids. However, the T_m and ΔH values of the dipentadecylmethylidene phospholipids are considerably lower. In the case of phosphatidylcholines, for instance, 1,2-dipalmitoyl- and 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine, ΔH values of 8.1 (16 carbons per chain) and 6.0 kcal/mol (14 carbons per chain), respectively, were observed (Table Ia).

This known chain length dependence of ΔH for the ester and ether lipids allows the calculation of an effective chain length for DPMPC which corresponds to about 13 carbon atoms. Thus, the ring carbon and the next two methylene groups in each chain are strongly restricted in their conformational freedom and do not contribute to the transition. Similar considerations hold also for phosphatidylethanolamines, whereas ether phosphatidic acids and DPMPA behave differently at pH 7. They have considerably lower ΔH values than the respective ester phosphatidic acids.

We now want to discuss the effect of changes in head group structure on the thermotropic behaviour. Increasing methylation of the head group of DPMPE leads to a linear decrease in T_m of 6.5°C per additional methyl group. The comparable values for dipalmitoyl phospholipids are 7.7 and for the dihexadecyl phospholipids 8.9°C (Table 1b). The increase in steric hindrance in glycerol backbone region in going from the diether through the diester to the dipentadecylmethylidene lipids obviously diminishes the influence of the polar groups on T_m. This is not an effect due to the reduced chain length of the dipentadecylmethylidene lipids because the influence of the polar groups on T_m is usually larger when the chains become shorter. For dimyristoyl phospholipids, for instance, ΔT_m between phosphatidylethanolamine and phosphatidylcholine is 27 as compared to 23°C for the dipalmitoyl phospholipids. A second example in this respect is the difference in T_m between phosphatidic acid and phosphatidylcholine. This amounts to 28°C for the dimyristoyl, 24°C for the dipalmitoyl and only 21°C for the dipentadecylmethylidene phospholipids.

Because of the excellent chemical stability of the dipentadecylmethylidene phospholipids in the alkaline pH region, we could also study the pH dependence of the transitions of DPMPA and DPMPE. The results obtained for DPMPA confirm our earlier observations for dimyristoyl phosphatidic acid and dihexadecyl phosphatidic acid that the transition temperature as well as the transition enthalpy decreases when the polar group become doubly charged [11]. The difference in ΔH between singly and doubly charged DPMPA, however, is considerably lower (approx. 1 kcal/mol) as compared to dimyristoyl phosphatidic acid (3.5 kcal/mol) and dihexadecyl phosphatidic acid (2.2 kcal/mol). The same trend is observed for the difference in T_m between the singly and doubly charged form, which is only 21°C for the dipentadecylmethylidene as compared to 25°C for the dihexadecyl phosphatidic acid. Concerning the ether phosphaticic acid, these lower T_m and ΔH values for the double charged form were attributed to an increase in the tilt angle of the hydrocarbon chains [10] and the impossibility of intermolecular hydrogen bonds being formed between the polar head groups [25]. The same phenomenon may also occur in DPMPA bilayers, although obviously to a lesser extent due to conformational restrictions in the glycerol backbone region.

In the alkaline pH region phosphatidylethanolamines behave very similarly to the phosphatidic acids. It was known before that the T_m value decreases when the ammonium group is deprotonated [24]. No systematic study of the pH dependence of the transition enthalpy was carried out, however. Our data show that also for phosphatidylethanolamines, ΔH decreases at high pH. This is probably correct for all phosphatidylethanolamines regardless of the kind of linkage of the hydrocarbon chains. This behaviour may be explained by an increase in the tilting of the hydrocarbon chains which lowers cohesion energies and thus ΔH. However, X-ray investigations have to be carried out to confirm this suggestion. In this context, it is interesting to note that phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid at pH 12 all have very similar transition temperatures and comparable transition enthalpies, regardless of whether these molecules are neutral or have one or even two negative charges, respectively. Therefore, when no intermolecular hydrogen bond between the head groups is possible, all phospholipids with the same chain length have almost the same transition temperatures. The normally higher T_m values for phosphatidylethanolamine and phosphatidic acid at neutral pH are thus in part due to intramolecular hydrogen bonds as already suggested before [4,25].

The T_m value of doubly charged DPMPA increases slightly with the square root of the salt concentration. This was also observed for the methyl ester of dimyristoyl phosphatidic acid and was attributed to changes in electrostatic free energy due to charge-screening effects [21]. Inserting the experimental value of $1°C/(mol/l)^{1/2}$ in the theoretical expression for the salt-induced increase in T_m derived by Träuble et al. [21] from a modified Gouy-Chapman theory of the electrical double layer gives a very low value for the change in molecular area at the transition. This is in general agreement with the results of X-ray investigations of dihexadecyl phosphatidic acid which give a value of 3.2 Å^2 for the change in area at the transition at pH 12 [23]. The salt depen-

dence of the ΔH values, however, is rather puzzling and cannot be explained by any simple arguments. The increase in ΔH up to 0.2 M NaCl may be due to charge-screening effects and/or changes in the hydration of the polar group which may also change the tilt angle of the chains. At still higher salt concentrations, the ΔH value decreases again. It may be that this is an electrostatic effect as predicted by the modified Gouy-Chapman theory of Träuble et al. [21]. However, the experimental variation of ΔH is far too large to be due solely to these changes in electrostatic energy, which would account for maximally 0.1 kcal/mol. This effect is not limited to double charged phosphatidic acid but can also be observed with the methyl ester of phosphatidic acid, although to a lesser extent (Blume, A., unpublished observations). Further studies will have to be carried out for a better understanding of this phenomenon.

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