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DIFFERENTIAL SCANNING CALORIMETRY OF DIPALMITOYL PHOSPHATIDYLCHOLINE ANALOGUES AND OF THEIR INTERACTION PRODUCTS WITH BASIC POLYPEPTIDES

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Summary

The thermotropic behaviour of dipalmitoyl phosphatidylcholine analogues with a varying number (n) of CH_2 groups between the phosphate and the quaternary ammonium has been investigated. The temperature (T_m) and the enthalpy (ΔH) of the phase transition are non-monotonous functions of the number of CH_2 groups. T_m oscillates between 40 and 45°C and ΔH between 7 and 13 kcal/mol for a variation of n between 2 and 11.

It is concluded that the hydrocarbon chains in the head groups do not penetrate the hydrocarbon region and do not contribute directly to the melting of the acyl chains. It is suggested that their length may affect the critical ballance between the attractive and the repulsive forces within the bidimensional lattice of the head groups.

Copolypeptides of lysine with phenylalanine do not appreciably affect the T_m but have a pronounced effect on ΔH of the lipid phase transition, which depends strongly on the ratio of the two amino acids in the polypeptide. The effect of copolypeptide of any defined composition on ΔH is also a non-monotonous function of the number of CH_2 groups in the phosphatidylcholine head group, but it does not parallel completely the oscilations in the T_m and ΔH of the pure lipids.

Introduction

The thermal analysis of lipids was initiated by Chapman [1] in the 1960's. It was reported that the temperature of the phase transition of the lipids depends upon the hydrocarbon chain length, their degree of unsaturation [1] and on

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Abbreviation: DPPC, dipalmitoyl phosphatidylcholine.

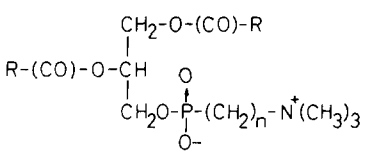
the type of the head group [2]. When dispersed in an excess of water, dipalmitoyl phosphatidylcholine (DPPC) has two reversible transitions, a small pretransition at 35°C and a main transition at 42°C. The pretransition was assumed to be associated with a change in the tilting angle of the hydrocarbon chains, with respect to the plane of the bilayer [3,4], whereas the main transition stems from the 'melting' of the hydrocarbon chains. If the DPPC is subjected to sonication, two transitions of comparable size are detected and those with a prolonged storage revert into the higher one (41°C) [5—8].

It was found in this laboratory [9] that phosphatidylcholine monolayers are less penetrable by substances such as polypeptides and proteins than most other phospholipids of identical hydrocarbon chain lengths. This behaviour was attributed to a bidimensional lattice of the phosphate groups with the quaternary ammonia in the cavities between them. The suggested structure is in agreement with the recent findings that the zwitterionic dipoles of the phosphatidylcholine form a small angle with the plane of the layer [10]. The accomodation of the quaternary ammonia in the cavities between the phosphate groups and its effect on the stability of the layer structure is expected to be a function of the distance between the phosphate and the positively-charged nitrogen atom determined by the number of the connecting CH_2 groups.

To check this hypothesis we carried out differential scanning calorimetry studies on dipalmitoyl phosphatidylcholine analogues with different chain lengths between the phosphate and the quaternary ammonium groups. Using this method we hoped to show how the gradual change in the polar part of the molecule influences the phase transition temperature and enthalpy. According to previous studies [11] basic polypeptides interacting with dipalmitoyl phosphatidylcholine affect the enthalpy of melting of the lipid without significant change in the melting temperature. We studied, therefore, also the influence of basic copolypeptides of lysine with phenylalanine on the thermotropic properties of the DPPC analogues with the different PO^- to N^+ distances.

Experimental

The synthesis of the 1,2-dipalmitoyl-*sn*-glycerol-3-phosphorylcholine (dipalmitoyl phosphatidylcholine, DPPC) and its analogues (Fig. 1) with stepwise increase in the number of CH_2 groups between phosphate and trimethylammonium is described elsewhere [12] and by Diembeck, W. and Eibl, H. (in preparation).



$R=(CH_2)_{14}CH_3$

$n=2$ to 11 and 16

Fig. 1. Chemical formula of the lipid.

The random copolymer of lysine : phenylalanine of narrow molecular weight and composition (2.1 : 1) was synthesized and fractionated on a Sephadex G 100 column by Dr. P. Lelkes in this laboratory. Its molecular weight, as determined by calibration of the elution profile and in the ultracentrifuge, was 70 000. The molar ratio lysine : phenylalanine (2.1 : 1) and the concentration were determined in an amino acid analyzer. The sequential polypeptide $(Lys\text{-}Phe\text{-}Lys)_n$ was synthesized by Dr. L. Muszkat in this laboratory.

The polylysine (molecular weight 70 000) and the random, unfractionated copolymer (lysine : phenylalanine, 1.2 : 1) were Miles Yeda products (Miles Yeda, Kiryat Weizmann, Rehovot).

The calorimetric measurements were performed on a DuPont 990 differential scanning calorimeter with cell base II equipped with a home-made cooling device. The calibrated mode was used with heating and cooling rates of 1, 2 and 5°C/min. The solid samples were weighed directly into the aluminum pans (approx. 2—4 mg), excess of water added, and then the pans were sealed hermetically and left overnight for equilibration. The ΔH values were calculated by integration over the two transition peaks.

The differential scanning calorimetry investigations of the interaction mixture between the phospholipids and the polypeptides were done on bath-sonicated phospholipids. The sonication was performed either on the lipids alone or on the respective mixtures with polypeptides. The sonication was usually done at concentrations of 100 mg/ml (45°C, 1 h) in a bath-type sonicator (Laboratory Supplies Company, Hicksville, N.Y., U.S.A.) so as not to affect the thermotropic properties of the lipids. Because of limited polypeptide solubility, in some cases the sonication was carried out in the presence of 40 mg/ml phospholipid. Since, in these cases, the thermotropic properties were affected (ΔH was lowered), we calculated the relative effect of the polypeptides on the ΔH.

The interaction products were prepared by mixing of appropriate amounts of the lipid suspension with a solution of the polypeptides (both at pH 7) to give a molar ratio of lysine : lipid of 1 : 1. The pH after dilution remained at 7 within 0.5 pH units. The interaction products were incubated for 1 h at 45°C, the sample was transferred into the aluminum pan and differential scanning calorimetry measurements were performed. The rest of the material was left overnight at room temperature, incubated for 1 h at 45°C and differential scanning calorimetry thermograms were obtained 24 h after preparation of the sample.

Results

Phase transition in pure lipids. Lipids wetted by a measured excess quantity of water produced thermograms which were identical (at least for the second and any further scan or after overnight incubation) with the thermograms of the dispersions produced in a vortex mixer. Thus, obtained results are presented in the following figures.

Figs. 2 and 3 present the endothermic (heating mode) and the exothermic (cooling mode) thermograms, respectively, of DPPC and of its analogues. As seen from Fig. 2, the pretransition in the melting curves typical for DPPC is

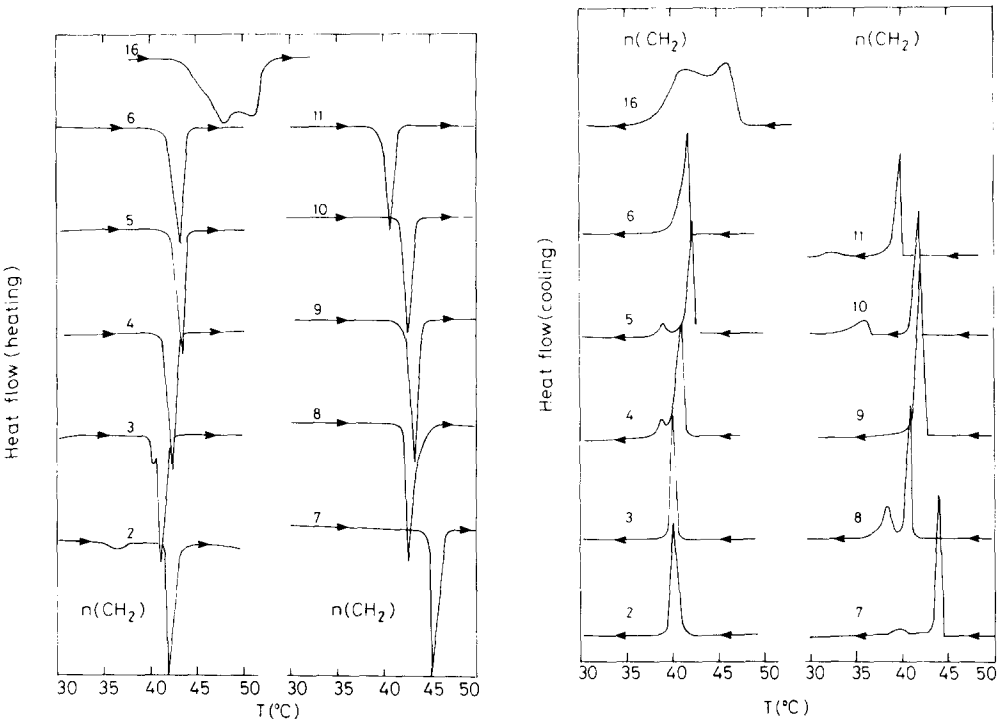


Fig. 2. The endothermic differential scanning calorimetry thermograms of dipalmitoyl phosphatidylcholine and its analogues. Scanning rate, 1°C/min.

Fig. 3. The exothermic differential scanning calorimetry thermograms of dipalmitoyl phosphatidylcholine analogues. Scanning rate, 1°C/min.

observed only in its C_3 analogue as a small shoulder at higher temperature, and is completely absent in the analogues with higher C values between PO^- and N^+. Above C_4 a small transition peak on the exothermic scan is seen. The secondary transitions are in every case at a lower temperature than the main transition. This secondary transition is not detected in C_6 (except of a shoulder) and C_9, whereas it appears for C_7, C_8, C_{10} and C_{11}. All the thermograms presented in Figs. 2 and 3 were obtained at cooling and heating rates of 1°C/min.

Fig. 4, a and b presents the temperature of melting and freezing (T_m, the temperature of the middle of the peak in the heating and the cooling mode) as a function of the number of CH_2 in the head groups. The values of T_m were measured at three different scan rates (5, 2 and 1°C/min) and extrapolated to an infinitely slow scan rate. The values of the endothermic (heating mode) T_m are always higher than of the exothermic ones, which produces a hysteresis loop. The difference between the exothermic and the endothermic T_m decreases with decreasing scan rate. However, linear extrapolation to scan rate zero is probably not adequate and even the extrapolated T_m values differ by 1—2°C, being higher for the heating mode. Nevertheless, the two curves in Fig. 4 run almost parallel showing minima at 3, 6 and 8 CH_2, and maxima at 5, 7 and 9 CH_2 groups between PO^- and N^+. At 11 CH_2 there is a marked decrease in T_m. It is interesting also to compare the even and odd numbered

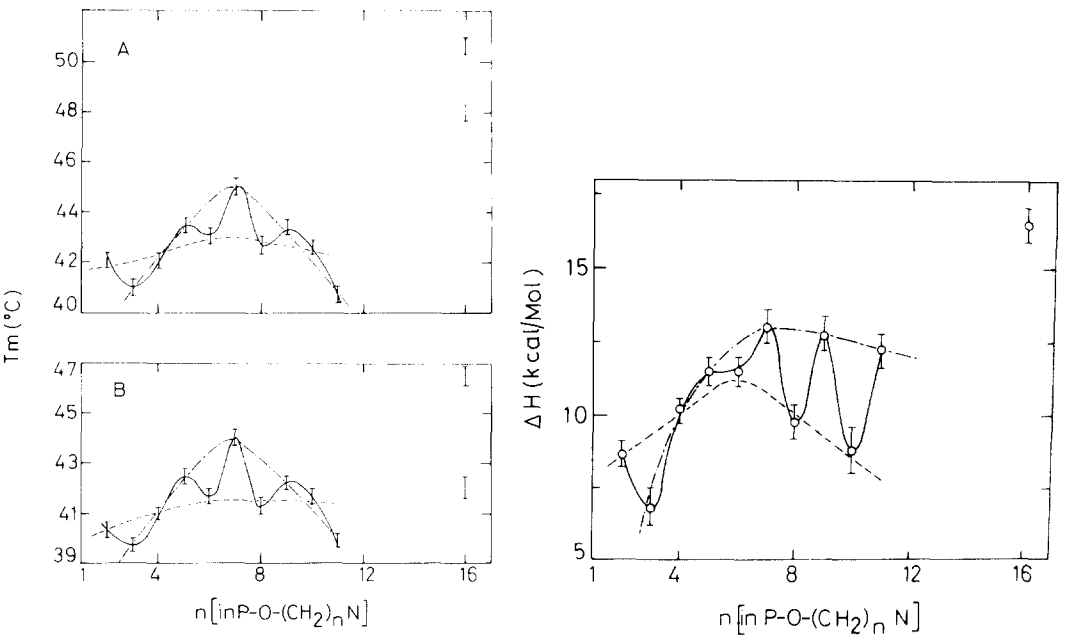


Fig. 4. A, the melting temperature (T_m endothermic); B, the freezing temperature (T_m exothermic) as a function of n, the number of CH_2 groups between PO^- and N^+. Curves connecting, even (- - - - -) and odd (- · - · -) values of n.

Fig. 5. The enthalpy (ΔH) of the phase transition (melting of freezing) as a function of n, the number of CH_2 groups between PO^- and N^+. - - - - -, even values; - · - · -, odd values of n.

series separately as done by the dashed lines. Each shows that T_m for the even numbered analogues is almost constant while the odd numbered one shows a hyperbolic curve with a maximum at C_7. We lack the phosphatidylcholine analogues between 12 CH_2 and 15 CH_2, but $-(CH_2)_{16}$ compound produces two transition peaks at higher temperatures (T_m (endothermic) = 46.5 and 48°C, T_m (exothermic) = 44.5 and 46.0°C).

The enthalpy (ΔH) of the phase transition calculated by integrating the heat flow peak is, as expected, identical in the heating and in the cooling mode. ΔH is presented in Fig. 5 as a function of the number of CH_2 groups between the phosphate and the quaternary ammonium. The variation of ΔH with the number of CH_2 is almost parallel to the variation of T_m. Only the ΔH of the $(CH_2)_{11}$ analogue is higher than that of $(CH_2)_{10}$, while its T_m is lower. Similarly to the T_m curves, here also nearly hyperbolic curves are obtained upon connecting the ΔH values of the dipalmitoyl phosphatidylcholine analogues containing even and odd numbers of CH_2 groups (dashed lines). The respective curves show maxima at $(CH_2)_6$ and $(CH_2)_7$. This behaviour seems to stop at number of (CH_2) groups between P and N larger than 11 since at $(CH_2)_{16}$ both T_m and ΔH values are higher than those of the shorter chain analogues.

Effect of interacting copolypeptides of lysine with phenylalanine on the phase transition of DPPC analogues. To assure good interaction between the lipid and the polypeptide, the lipid was sonicated before adding the poly-

peptide. The sonicated lipid was then divided into two equal parts, to one of which an equimolar (based on the amount of positive charges of lysine residues) portion of the polypeptide was added. Differential calorimetric scans of the lipid/polypeptide mixtures and of the lipid controls were carried out in consecutive runs. The polypeptide affected the ΔH but not the T_m values. The effect of the polypeptides on the relative change in ΔH was reasonably reproducible, even though the thermograms of the lipids sonicated at low concentration were affected by the sonication in a not completely reproducible manner. The thermograms were dependent not only on the sonication time and intensity but also on the concentration of the lipid during sonication. Under our working conditions only sonication at concentrations below 40 mg/ml changed the thermotropic behaviour producing in some cases double transition peaks with lower ΔH values on the two peaks [6]. But even in these cases the relative change in ΔH upon addition of the polypeptide remained unaffected.

The variation in ΔH depends on the P to N distance in the head group, as well as on the composition of the polypeptide (Fig. 6). As reported in a previous publication [11], a 1.2 : 1 lysine/phenylalanine copolypeptide lowers the transition enthalpy of DPPC. The same results have been obtained here with a random copolypeptide of a similar composition but a higher molecular weight. Polylysine causes a negligible or no increase in ΔH, as do copolypeptides containing a high lysine : phenylalanine ratio. Even a sequential copolypeptide $(Lys\text{-}Phe\text{-}Lys)_n$ causes only a negligible change in ΔH of the regular DPPC. A random copolypeptide of about the same composition (Lys/Phe = 1.8—2/1) causes an increase in the ΔH of this phospholipid. At lysine to phenylalanine ratio around 1.3 : 1 the effect of the copolypeptide on ΔH of

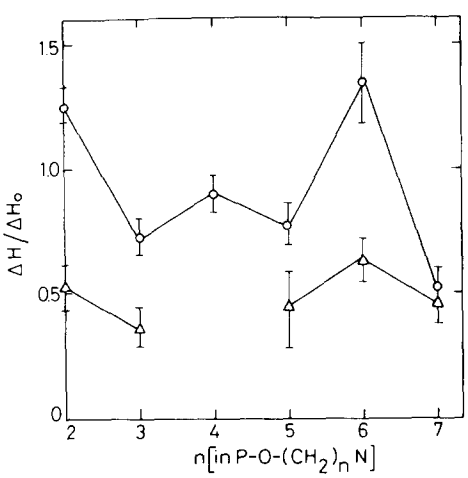


Fig. 6. $\Delta H/\Delta H_0$, relative change of transition enthalpy of the interaction mixtures formed by adding equivalent amounts of copolypeptides to dipalmitoyl phosphatidylcholine analogues with different number of CH_2 between N^+ and PO^-. Circles, random copolypeptide (Lys : Phe approx. 2 : 1), M_r = 70 000; triangles, random copolypeptide (Lys : Phe approx. 1.2 : 1), high undetermined molecular weight.

DPPC has to go through zero since upon further increase of phenylalanine in the copolypeptide a decrease in ΔH is observed.

Two copolypeptides, one of a narrow composition of lysine/phenylalanine (1.9 ± 0.1) : 1 and of narrow molecular weight distribution (70 000) and a high molecular weight copolymer with a lysine : phenylalanine ratio (1.2 : 1) were selected for comparing their influence on the phase transition of different dipalmitoyl phosphatidylcholine analogues. The results are summarized in Fig. 6, where the relative variation in ΔH upon interaction with an equimolecular amount (based on lysine residues) of copolypeptide is presented for the different phosphatidylcholine analogues (with $(CH_2)_2$ up to $(CH_2)_7$ between N and P in the head group). A periodic variation in ΔH is observed. The periodicity is not completely parallel with the periodicity observed in the variation of the ΔH and T_m of the pure lipids. However, polypeptides of different compositions seem to give parallel, even though shifted, effects on ΔH. The effect on T_m is, in every case, very marginal and does not exceed 1—1.5° C.

Discussion

The transition temperature of dimyristoyl phosphatidylcholine is about 19° C lower than that of dipalmitoyl phosphatidylcholine, while the transition temperature of distearoyl phosphatidylcholine is about 20° C higher. Thus, each additional CH_2 group per hydrocarbon chain involved directly in melting causes an increase in T_m of about 10° C. An increase in the number of CH_2 groups between the phosphate and the quaternary ammonium in the head group has only a small varying effect on the transition temperature. One can conclude from this that the hydrocarbon chains up to $(CH_2)_{11}$ in the head group are not involved directly in the melting process. They seem, however, to influence the melting process through their effect on the average distance between the head groups in the polar layer. The more condensed the polar layer, the larger the fraction of the hydrocarbon chains within the hydrophobic core taking part in the cooperative phase transition and the higher its temperature and enthalpy. The P to N distance given by the number of CH_2 groups can influence the average distance between the polar groups by the way it allows accomodation of the quaternary ammonium between the negatively-charged phosphate groups within the polar layer. The area per molecule (polar group) for phosphatidylcholine is between 45 and 60 Å^2 below and above the phase transition, respectively. If the lipids are in a triangular array the average distances between the negative charges on the phosphate are 7.3 and 8.4 Å, respectively. Yaegle et al. [13] deduced from the results of their nuclear Overhauser effect study that the average distance between the nine protons of the quaternary ammonium residue and the interacting phosphate neighbour is 3.2 Å. Model studies indicate that the distance of the closest approach between the positive charge on the quaternary ammonium and the negative charge on the interacting phosphate may be also between 3 and 3.5 Å. Space-filling models show also that a phosphatidylcholine head group cannot be accomodated in one plane within an area less than 78—80 Å^2. At an area of 45—60 Å^2 per molecule the quaternary ammonium is bound to be 2—3 Å above the plane of the negative phosphate charges. These restrictions, together with the length of the hydrocarbon chain

between the ammonium and the phosphate residue, will determine the sum of electrostatic repulsion and attraction forces of the charges of identical and opposite signs. The residual attractive or repulsive forces are then balanced by configurational strains and hydrophobic forces to obtain the equilibrium accomodation of the lipid molecules in the surface.

The PO^- to N^+ distance in the ordinary phosphatidylcholine ($(CH_2)_2$) is 6.8 Å in the stretched out all-*trans* configuration. With every additional CH_2 residue this distance increases by about 1.27 Å. *Gauche* configurations reduce these distances considerably and the probability of *gauche* configurations increases with the number of CH_2 groups. It can be shown that the balance of electrostatic attraction and repulsion depends strongly on the location of the positive group with respect to couples of nearest neighbours or next nearest neighbour negative groups. This is shown in Fig. 7 where rows of zwitterionic groups with progressively increasing distance between the positive and negative residues in the same molecule. The positive charges of the first row of molecules are short of reach of the negative charges of the next row of molecules. One would expect attraction here. In reality the balance of repelling and attractive forces between the two rows of molecules depends also on the distance between the rows and on the lift of the positive residues with respect to the negative plane. For example, if the average distance between the nearest neighbour charges is 8.4 Å the distance between the positive and the negative charges in the same molecule is 6.8 Å and the lift of the positive with respect to the negative charges is 2.5 Å, then the electrostatic repulsive forces are nearly balanced. Increase in the lift enhances repulsion whereas increase in the distance between the molecules will enhance attraction causing molecules to snap back. Increase in the distance between the positive and negative charges will enhance repulsion between the rows of molecules until attraction to the next nearest negative charges overtakes. Thus, by increasing the distance

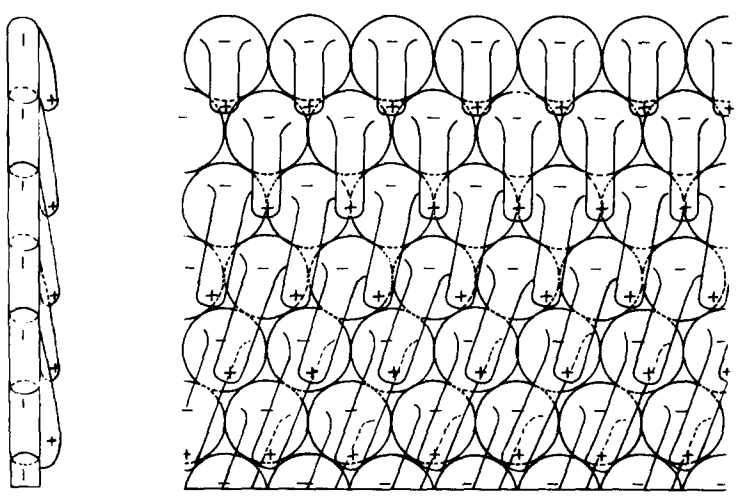


Fig. 7. Schematic presentation of two-dimensional arrangement of the head groups of phosphatidylcholine analogues. The N^+ to PO^- distance increases in consecutive rows. Top view and side view.

between N^+ and PO^- attraction and repulsion can alternately dominate the arrangement of the molecules. These alternating attraction and repulsion forces do not change the average area per molecule a great deal since they are counteracted by van de Waals and hydrophobic forces. Indeed monolayer compression curves do not show any discernible periodic variations in areas [12]. They may suffice, however, for periodically altering the heat and the temperature of transition. It should be noted that 'phase transitions' obtained with probes do not show any periodic temperature variations [12].

One could also draw an analogy from the aliphatic hydrocarbons or alcohols where the melting point is not a linear function of the number of CH_2 groups. The line combining the melting temperatures of the compounds containing an even number of methylene groups lies above that of the odd ones. It was, therefore, reasonable to combine also the transition temperatures and heats of the lipids for the even and odd numbers of CH_2 groups between PO^- and N^+. It is obvious from Figs. 4 and 5 that simple hyperbolic up to straight lines are obtained, except in the present case the two lines cross over.

As seen from Figs. 2 and 3 only the C_2 and C_3 analogues show pretransitions as described by others [3]. Obviously, for C_2 and C_3 the so-called tilt is seen separately from the melting of the chain during heating. However, in the other cases either the tilting merges completely with the melting or it just cannot be resolved experimentally during heating. The event reminiscent of the tilting phenomenon can, however, be observed in most of the phospholipids above C_4 during cooling.

The relative sizes of the secondary transition peaks vary in size and so does the temperature difference (up to 7° C) from the main transition.

Basic polypeptides compete with the quaternary ammonia of the choline for interaction with negative sites. The competitive capacity of the polypeptides is assisted by the hydrophobic interactions of the phenylalanine residues. This combined electrostatic-hydrophobic interaction may have an adverse effect on the thermotropic properties of the phosphatidylcholine analogues. While the hydrophobic penetrating interaction causes a lowering in the transition enthalpy, the increased concentration of charged residues near the lipid surface may bring about an increase in the enthalpy and may also cause a small increase in T_m. The total effect seems to be a complex function of the hydrophobicity and charge (ratio of phenylalanine to lysine) of the polypeptide, as well as of the number of CH_2 groups between PO^- and N^+ in the lipid. For any particular phosphatidylcholine analogue ΔH increases only negligibly upon interaction with polylysine or with copolypeptides with a high Lys/Phe ratio. The increment in ΔH is enhanced by an increasing amount of phenylalanine in the copolypeptide until a maximum is reached at a certain ratio of phenylalanine to lysine. However, the sequence of the amino acids in the polypeptide may also be of importance. The sequential polypeptide $(Lys\text{-}Phe\text{-}Lys)_n$ where -Phe-Phe- sequences are completely eliminated seems to have a considerably smaller effect on ΔH than the random copolypeptide of a similar composition. Upon further enrichment of the polypeptide with phenylalanine the effect of hydrophobic penetration takes over and ΔH starts decreasing to values below those for pure lipids. The slope of this dependence on the composition of polypeptides is expected to differ for the different phosphatidylcholine analogues.

It is, therefore, not surprising that the effect of the polypeptide of one single composition on ΔH of the different lipids does not follow exactly the behaviour of the pure lipids. On the countrary, bearing in mind that phase transition temperatures, as detected by probes, do not show any periodic variation one may wonder why interaction products with polypeptides do. Further studies of the correlation between head group composition and structural stability in the intact state and upon interaction are required and are planned.

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References

1 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304—367
2 Vaughan, D.J. and Keough, K.M. (1974) FEBS Lett. 47, 158—161
3 Rand, R.P., Chapman, D. and Larsson, K. (1975) Biophys. J. 15, 1117—1124
4 Janiak, M.J., Small, D.M. and Graham-Shipley, G.G. (1976) Biochemistry 15, 4575—4580
5 Suurkuusk, J., Lentz, B.R., Barenholz, Y., Biltonen, R.L. and Thompson, T.E. (1976) Biochemistry 15, 1393—1401
6 Papahadjoponlos, D. Hui, S., Vail, W.Y. and Poste, G. (1976) Biochim. Biophys. Acta 448, 245—264
7 Kantor, H.L., Mabrey, S., Prestegard, J.H. and Sturtevant, J.M. (1977) Biochim. Biophys. Acta 466, 402—410
8 Van Dijck, P.W.M., de Kruijff, B., Aarts, P.A.M.M., Verkleij, A.J. and de Gier, J. (1978) Biochim. Biophys. Acta 506, 183—191
9 Miller, I.R. and Rishpon, J. (1977) in Electrical Phenomena at the Biological Membrane Level (Roux, E., ed.), pp. 93—110, Elsevier Scientific Publishing Company, Amsterdam
10 Seelig, J. and Gally, H.U. (1976) Biochemistry 15, 5199—5204
11 Bach, D. and Miller, I.R. (1976) Biochim. Biophys. Acta 433, 13—19
12 Diembeck, W. (1976) Ph.D. Thesis, University of Braunschweis, West Germany
13 Yaegle, P.L., Hutton, W.C., Huang, C.H. and Martin, R.B. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3477—3481