CHANGES IN PHASE TRANSITIONS OF PHOSPHATIDYLETHANOLAMINE– AND PHOSPHATIDYLCHOLINE–WATER DISPERSIONS INDUCED BY SMALL MODIFICATIONS IN THE HEADGROUP AND BACKBONE REGIONS

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Received 3 August 1974

1. Introduction

Phosphatidylcholines (PC's) and phosphatidylethanolamines (PE's), in general, comprise the major lipids of many membranes, but only recently has information become available on the phase behaviour of these compounds in model membranes. It has been found with saturated phospholipids, in water, that gel to liquid crystalline (l.c.) phase transition temperatures of phosphatidylethanolamines are substantially higher than those for phosphatidylcholines, and that PC's display a small endotherm (pretransition) a few degrees below the main methylene chain transition which is absent in PE's [1–5]. On the other hand, transition enthalpies for dimyristoyl PE and dimyristoyl PC are almost the same [5]. These findings have been extended to determine the effect of minor changes in the headgroup and glycerol 'backbone' region of a series of *N*-methylated PE's and their diether analogues on transition temperatures and heats and the appearance of a pretransition. Methylation of the PE headgroup was accompanied by a corresponding reduction in temperature and diether analogues of the phospholipids had higher transition temperatures than the corresponding diester compounds. Transition heats did not change systematically with either methylation or the substitution of ether for ester bonds. Only dipalmitoyl PC (DPPC) and its diether analogue (DHPC) showed pretransitional endotherms.

2. Materials and methods

The following lipids were obtained from Calbiochem, La Galla, California: dipalmitoyl PE (DPPE), dihexadecyl PE (DHPE), *N*-methyl dipalmitoyl PE ($N(CH_3)$DPPE), *N*-methyl dihexadecyl PE ($N(CH_3)$DHPE), *N,N*-dimethyl dipalmitoyl PE ($NN(CH_3)_2$DPPE), *N,N*-dimethyl dihexadecyl PE ($NN(CH_3)_2$DHPE), dipalmitoyl PC (DPPC) and dihexadecyl PC (DHPC). All gave only one spot on thin layer chromatography in solvent systems for both phospho- and neutral lipids [6].

Lipid dispersions were made in deionized glass distilled water by heating the lipid–water (approx. 1/2, w/w) mixtures 10–15°C above the expected gel to l.c. transition temperature and mixing on a vortex mixer. Thermal analyses were performed on a Perkin-Elmer DSC-2 differential scanning calorimeter. Thermograms were obtained at a rate of 10°C min and a sensitivity of 10 mcal/sec full scale with air as the reference. After thermal analyses, samples were quantitatively extracted in chloroformmethanol (1/1, v/v) for phosphate analyses [7]. Three or four dispersions of each lipid were analyzed. The DSC-2 was calibrated using pure indium (Perkin–Elmer), 99 mole % benzene (Fisher Scientific, Montreal) and 99.5% stearic acid (Fluka, Buchs). Areas on the thermograms were determined using a planimeter.

3. Results and discussion

Tracing of the thermograms for the diester and diether series of lipids are shown in fig. 1. The main chain transition occurs at progressively lower temperatures with increasing methylation of the headgroup both for the diester phospholipids and their diether analogues. Temperatures are reduced by 7.7–8.7°C for each methyl group. The diether

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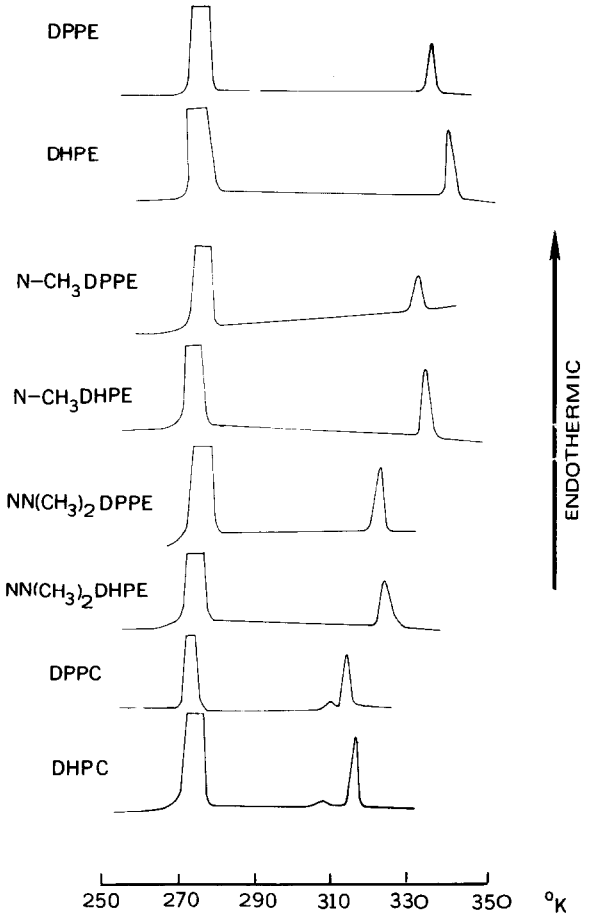

Fig. 1. Tracings of thermograms of water dispersions of dipalmitoyl and dihexadecyl PE's, PC's and their *N*-methylated intermediates. Abbreviations given in text. The low temperature endotherm is due to water.

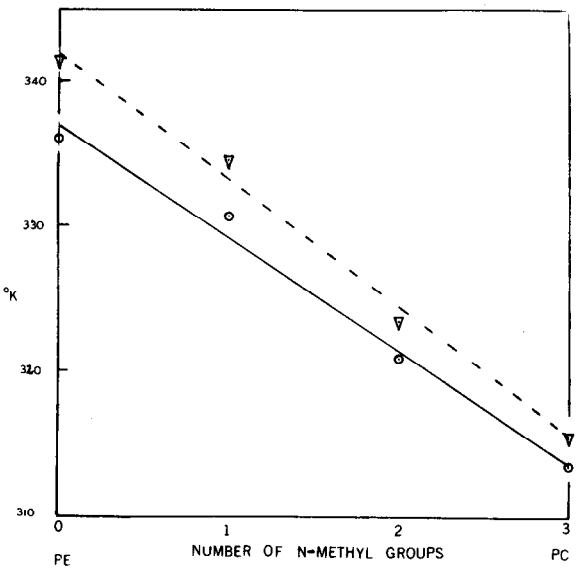

Fig. 2. Variation in transition temperatures with headgroup methylation for dipalmitoyl (o–o–o) and dihexadecyl (△–△–△) lipids.

analogues, display consistently higher transition temperatures (by 2.4 to 5.3°C) than their corresponding diester phospholipids. Closer packing in the plane of the bilayer of the diether lipids, because of the absence of the carboxyl groups in the glycerol 'backbone' regions, would be consistent with the observed transitions. Thus the presence of ether bonds in lipids [8] may provide for a method for the regulation of fluidity in some membranes.

As can be seen in fig. 2, for both series of lipids the main methylene chain transition temperature shows an inverse linear relationship with the extent of headgroup methylation. The simplest explanation for this behaviour would be that increased bulk (by methyl group introduction) in the headgroups of the phospholipids allows for a decreased packing density and thus lower transition temperature. It is also possible that increased methylation changes the orientation between the headgroups and the plane of the bilayer with consequent changes in packing density. Monolayer studies [9–11] have indicated that PE's are more closely packed than PC's with the limiting areas per molecule for PE's being approximately 4 Å^2 less than for analogous PC's. Although all the lipids used would be expected to be zwitterionic over a broad pH range, Phillips et al. [12] have pointed out that charge neutralization may occur in PE's but not in PC's because of differences in headgroup orientation with the bilayer in the two lipid–water systems. They suggest that the PC headgroups are oriented normal to the bilayers whereas PE headgroups may be oriented tangential to the bilayers or interdigitated in a translamellar fashion. Whatever the packing arrangements of the headgroups, it can be seen from figs. 1 and 2 that very small changes in the headgroup or backbone regions can lead to substantial changes in the gel to l.c. transition temperature.

An examination of fig. 1 shows that only DPPC and

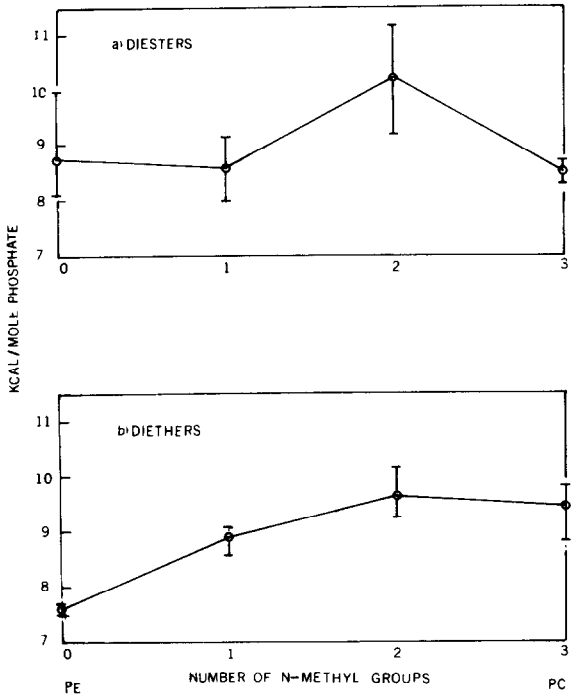

Fig. 3. Variation of main transition heats with methylation of the headgroup in dipalmitoyl (a) and dihexadecyl (b) lipids. The vertical lines indicate the range of values measured.

DHPC display the pretransitional endotherm characteristic of saturated lecithins. Alternative explanations of a headgroup–water rearrangement [1,5] or a change in the packing arrangement of the hydrocarbon chains [2] have been offered for the presence of the pretransition. The present findings are consistent with either interpretation. It is interesting to note, however, that even though the presence of ether groups instead of esters in the backbone appears to lead to more tightly packed methylene chains (the main chain endotherms are higher for the diethers than the diesters), the pretransition in DHPC occurs at a slightly lower temperature (32.8°C) than that for DPPC (34.6°C). An examination of ditetradecyl PC and dioctadecyl PC may help shed further light on this point. It is of consequence to note that a discernible pretransition is unique to the presence of the choline headgroup. Examination of $N,N(CH_3)_2$DPPE dispersions under conditions of increased sensitivity (10-fold) and a 10-fold slower programming rate failed to reveal any evidence of a pretransition. This still does not entirely preclude the possibility of endotherms for two processes occurring almost simultaneously.

Transition heats (fig. 3) show complex variation with methylation and diether substitution. The diethers (fig. 3b) show a general increase in transition enthalpies with methylation. The diester compounds, (fig. 3a) however, do not follow this pattern with the enthalpy of the DPPC main transition being approximately 1.6 kcal/mole lower than that for $N,N(CH_3)_2$DPPE. The main chain transition enthalpy (8.5 kcal/mole phosphate) from DPPC is in agreement to that found before by Phillips et al. [13] and Chapman et al. [5] but lower than the value found by Hinz and Sturtevant [2]. Applications of the type of correction used by Hinz and Sturtevant [2] failed to significantly alter the heat of the main transition. The heats for the choline containing lipids are approximately equal to or higher than those for corresponding PE's. Similar findings were made with the dimyristoyl derivations of PE and PC [5]. These observations, taken together with the observations cited above which indicate closer packing of PE's than PC's, would be consistent with a different packing arrangement of methylene chains in PC's and PE's. Differences between transition heats of the diesters and corresponding diether compounds are not systematic. Further work on analogues with different chain lengths may help to establish some pattern in these.

Acknowledgements

This work was supported by the Medical Research Council of Canada (MA-5036). D.J.V. thanks the Canada Department of National Health and Welfare for a RODA summer studentship. This work will form part of an M.Sc. thesis to be submitted to Memorial University of Newfoundland by D.J.V.

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