Biochimica et Biophysica Acta, 733 (1983) 111–116
Elsevier

BBA 71796

MONOLAYER AND CALORIMETRIC STUDIES OF PHOSPHATIDYLCHOLINES CONTAINING BRANCHED-CHAIN FATTY ACIDS AND OF THEIR INTERACTIONS WITH CHOLESTEROL AND WITH A BACTERIAL HOPANOID IN MODEL MEMBRANES

ELMAR KANNENBERG [a], ALFRED BLUME [b], RONALD N. McELHANEY [c] and KARL PORALLA [a]

[a] *Institut für Biologie II, Mikrobiologie I, Universität Tübingen, D-7000 Tübingen,* [b] *Institut für Physikalische Chemie II, Universität Freiburg, D-7800 Freiburg (F.R.G.) and* [c] *Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 (Canada)*

(Received January 31st, 1983)

Key words: Phosphatidylcholine; Branched-chain fatty acid; Cholesterol; Hopanoid; Phase transition; (Model membrane)

Although methyl iso- and anteiso-branched fatty acids occur widely in the membrane lipids of prokaryotic microorganisms, relatively little is known about the physical properties of phospholipids containing these fatty acids. We report here a monolayer and differential scanning calorimetric characterization of several synthetic phosphatidylcholines containing branched-chain fatty acids, and describe the interactions of these phospholipids with cholesterol and with a bacterial hopanoid. We find that monolayers as well as bilayers of methyl isobranched- and especially of methyl anteisobranched-fatty-acid-containing phosphatidylcholines exhibit a reduced solid-to-fluid phase transition temperature in comparison with linear saturated fatty acid-containing phosphatidylcholines of comparable chain length. We also find that the liquid-condensed or gel states of branched-chain fatty acid-containing phosphatidylcholines are partially disordered relative to those of phospholipids containing linear saturated fatty acids, although the presence of a methyl branch has only a small effect on hydrocarbon chain packing in the liquid-expanded or liquid-crystalline states. The presence of cholesterol was found to produce a marked condensation of liquid-expanded films and a small condensation of liquid-condensed films, whether the phosphatidylcholine contained linear or branched-chain fatty acyl constituents. The presence of a bacterial hopanoid produced similar, although slightly smaller, monolayer-condensing effects, indicating that these compounds may perform a cholesterol-like function in bacterial membranes.

Introduction

Methyl iso- and anteiso-branched saturated fatty acids occur widely as components of the membrane lipids of prokaryotic microorganisms [1]. In addition, branched-chain fatty acids are able to support the growth of several fatty acid-auxotrophic microorganisms in which these fatty acid classes do not naturally occur [2–4]. Model membranes [5,6] composed of di-isoacyl or di-anteisoacyl phosphatidylcholines (PC), and natural membranes [7–9] enriched in phospholipids containing branched-chain fatty acids, exhibit decreased gel to liquid-crystalline phase-transtion temperatures in comparison to membranes containing unbranched saturated fatty acids. This had led to the suggestion that phospholipids containing branched-chain fatty acids, and particularly methyl anteisobranched fatty acids, act as 'mem-

Abbreviations: PC, phosphatidylcholine(s); THBH, 1,2,3,4-tetrahydroxypentane-29-hopane; DPPC, dipalmitoylphosphatidylcholine; DHPC, 1,2-diheptadecanoylphosphatidylcholine; DIHPC, 1,2-diisoheptadecanoylphosphatidylcholine (15-methylhexadecanoyl-); DAHPC, 1,2-dianteisoheptadecanoylphosphatidylcholine (14-methylhexadecanoyl-).

brane fluidizers', just as to phospholipids containing *cis*-unsaturated fatty acyl groups [10]. Despite their wide distribution and experimental usefulness, relatively little is known about the physical properties of branched-chain fatty acid-containing phospholipids. For this reason, we initiated monolayer and differential scanning calorimetric (DSC) studies of model membranes composed of synthetic PCs containing methyl iso- or anteiso-branched fatty acids. The effect of cholesterol and of a bacterial hopanoid on the properties of monolayers and bilayers composed of these synthetic branched-chain PCs was also investigated. Some results of these studies are presented here.

Materials and Methods

The hopanoid 1,2,3,4-tetrahydroxypentane-29-hopane (THBH) was obtained from *Bacillus acidocaldarius* as described previously [11]. Descriptions of the synthesis and purification of DPPC, DHPC, DIHPC and DAHPC have already been published [5,6,12]. Monolayer experiments were carried out exactly as described by Blume [13]. The DSC analyses of the thermotropic phase behavior of aqueous multilamellar dispersions of the individual PCs were performed with a Microcal MC-1 Differential Scanning Microcalorimeter (Microcal Inc., Amherst, MA, U.S.A.) operating at a heating scan rate of 20 K/h, while the corresponding analyses of DPPC-THBH dispersions were performed with a Privalov Calorimeter [14].

Results and Discussion

In Fig. 1, the molecular areas occupied by monolayers of DPPC, DIHPC and DAHPC are plotted as a function of temperature at a constant lateral surface pressure of 25 dyn/cm. At this surface pressure, the transition from the liquid-condensed to the liquid-expanded state is half complete at temperatures of 32.5°C, 21.3°C and about 0°C (estimated) for DPPC, DIHPC and DAHPC, respectively. At a temperature 10°C above their measured or estimated phase transition temperatures, the molecular areas occupied by all three PCs are nearly comparable (about 63 Å^2), indicating a similar state of acyl chain packing in the fluid state. At a temperature 10°C below their phase transition temperature, however, the DPPC molecules occupy a much smaller area (51 Å^2) than do the molecules of DIPHC (54 Å^2) or

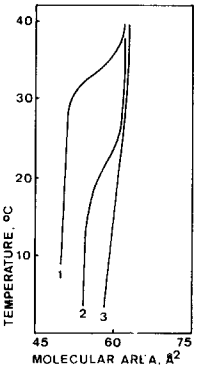

Fig. 1. Isobars at a surface pressure of 25 dyn/cm of monolayers of DPPC (1), DIHPC (2) and DAHPC (3).

DAHPC (about 56–58 Å^2), indicating that PCs containing branched-chain fatty acyl groups are significantly more disordered in the solid state than are PCs containing unbranched saturated hydrocarbon chains.

In Fig. 2, the average molecular areas occupied by monolayers composed of varying proportions of cholesterol and either DPPC, DIHPC or DAHPC are presented. The lateral surface pressure was again held constant at 25 dyn/cm and measurements were made at either 40°C or at a reduced temperature (see legend to Fig. 2). Since the area occupied by the rigid cholesterol molecules is almost temperature-invariant, one can see that in the expanded state which exists at 40°C, cholesterol markedly reduced the molecular area occupied by all three PCs in comparison to the molecular areas that these phospholipids would occupy in the absence of cholesterol at this temperature. However, the magnitude of the condensation produced by cholesterol levels of 25–50 mol% appears to be greatest for the DPPC and smallest for the DAHPC monolayer, perhaps reflecting the fact that this sterol exerts its maximum condensing effect at temperatures near the phase transition temperature of the phosphatidylcholine film [15]. At the reduced temperatures, where the DPPC and DIHPC (but not the DAHPC) monolayers exist in the liquid-condensed state,

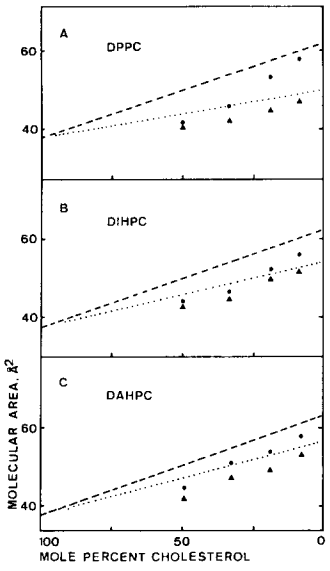

Fig. 2. Effect of cholesterol on the molecular areas of different PCs (A, DPPC; B, DIHPC; C, DAHPC) at varying molecular ratios of cholesterol/PC at a surface pressure of 25 dyn/cm. — — —, calculated areas at 40°C; · · ·, calculated areas at the lower temperature; ●, measured areas at 40°C; ▲, measured areas at the lower temperature. Lower temperature for DPPC = 15°C, for DIHPC = 10°C and for DAHPC = 4°C. The standard error margin is ± 1 Å².

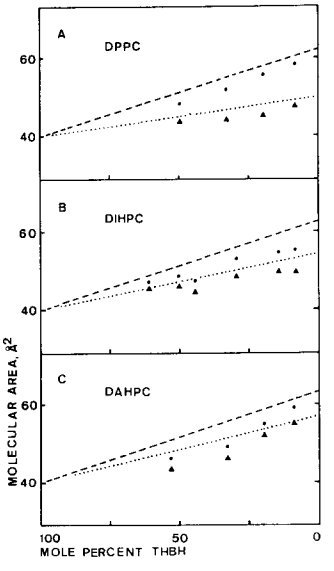

Fig. 3. Effect of THBH on the molecular areas of different PCs (A, DPPC; B, DIHPC; C, DAHPC) at varying molecular ratios of THBH/PC at a surface pressure of 25 dyn/cm. Conditions and abbreviations are the same as in Fig. 2.

cholesterol also produces a reduction in PC molecular area, although the magnitude of this reduction is considerably less than observed at higher temperatures, where these PC films exist in the liquid-expanded state. The finding that mixed monolayers of cholesterol and DPPC exhibit a condensation below their phase transition temperatures has been previously reported by other workers [16,17]. This effect may be due to a cholesterol-induced rearrangement of PC packing in the monolayer film, in which the fatty acyl chains become more nearly perpendicular to the film plane in the presence of cholesterol. A conformational change in the phosphatidylcholine headgroup may be important of this cholesterol-induced rearrangement, since mixed films of cholesterol and dimyristoylphosphatidic acid do not exhibit condensation at temperatures below the phase transition temperature [17].

In Fig. 3, the average molecular areas occupied by monolayers composed of varying proportions of THBH and either DPPC, DIHPC or DAHPC are presented, the measurements being made under the same conditions as in Fig. 2. The results obtained with this bacterial pentacyclic triterpenoid are essentially identical to those obtained with cholesterol, in that THBH significantly condensed all three monolayers above their phase transition temperatures and less markedly condensed the DPPC and DIHPC monolayers below their phase transition temperatures. However, the condensing effect of THBH is usually somewhat smaller than that produced by cholesterol under similar conditions. These findings support the suggestion that hopanoids may function to regulate the fluidity of the lipids in prokaryotic membranes in a manner analogous to that of cholesterol in eukaryotic membranes [11,18–20].

We also studied the thermotropic phase behaviour of model bilayer membranes composed of DHPC, DIHPC or DAHPC by high-sensitivity DSC; DHPC rather than DPPC was selected here so that all three PCs would have the same total number of carbon atoms in their fatty acyl chains, rather than the same 'effective' chain lengths. As can be seen in Fig. 4, DHPC exhibited two endotherms on heating. The broader, lower-energy

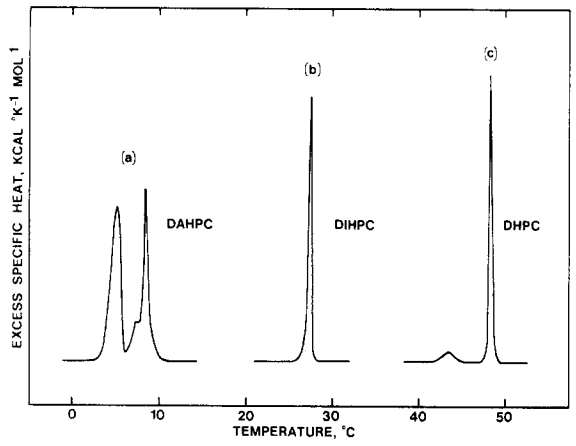

Fig. 4. High-sensitivity DSC traces illustrating the thermotropic phase behaviour of multilamellar dispersions of (a) DAHPC, (b) DIHPC and (c) DHPC. The peak areas are not drawn to scale.

transition occurring at 43.3°C is the pretransition, which is associated with a gel state packing rearrangement of the hydrocarbon chains, while the sharper, higher-energy main transition occurring at 48.2°C is due to the cooperative melting of the fatty acyl chains [21–23]. Also, DIHPC usually exhibits two endotherms, a sharp, energetic endotherm at 27.6°C, presumably due to the cooperative hydrocarbon melt, and a broad, less energetic endotherm (not shown in Fig. 4) centred near 10°C. The position and enthalpy of this lower-temperature transition, the physical basis of which is presently unknown, varied markedly with sample preparation, thermal history and heating rate. DAHPC always exhibited two relatively broad endotherms, centered at 5.2 and 8.3°C, the higher-temperature peak having a small low-temperature shoulder. The physical basis for this complex thermotropic behaviour is also not currently understood. The calorimetric enthalpies of the main transitions were DHPC = 8.8 ± 0.1, DIHPC = 8.1 ± 0.3 and DAHPC = 6.3 ± 0.4 kcal/mol (the latter provisionally taken as the sum of the areas of both peaks). The corresponding entropy of transition values were DHPC = 27.4, DIHPC = 26.9 and DAHPC = 22.9 cal/K per mol. The lower gel to liquid-crystalline phase transition temperatures, and the decreased transition enthaplies and entropies of the branched-chain PCs, particularly the anteiso-branched PC, again suggest that these lipids have higher energies and decreased degrees of order in their gel states compared to DHPC.

The thermotropic phase behaviour of model bilayer membranes composed of DPPC plus various amounts of THBH was also studied by high-sensitivity DSC. Some representative DSC traces are presented in Fig. 5. One can see that the presence of increasing quantities of this bacterial hopanoid abolishes the pretransition endotherm and decreases the cooperativity and enthalpy of the main chain-melting endotherm of DPPC, just as has previously been reported for cholesterol-DPPC mixtures (see Refs. 21 and 22). These results further confirm that cholesterol and THBH have qualitatively similar effects on the thermotropic phase behaviour of phospholipid model membranes.

The liquid-condensed to liquid-expanded phase transition temperatures observed for PC monolayers in the present investigation are some 7–9°C lower than the calorimetrically determined gel to liquid-crystalline phase transition temperatures of the corresponding PC bilayers observed in the present and in previous studies [5,6]. These differences in phase transition temperatures are probably due primarily to the lower lateral surface pressure utilized in the monolayer study (25 dyn/cm) as compared to the intrinsic lateral surface pressures characteristic of PC bilayer vesicles in water (estimated at 30–35 dyn/cm, see Refs. 13,

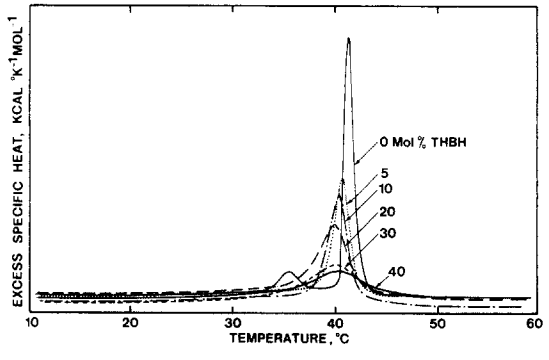

Fig. 5. High-sensitivity DSC traces illustrating the thermotropic phase behaviour of multilamellar dispersions of DPPC containing 0–40 mol% THBH. The peak areas are drawn approximately to scale.

24). It should be noted, however, that the relative differences between the phase transition temperatures of the various PCs studied here are quite similar in both the monolayer and bilayer membranes, confirming that the presence of a methyl branch in the iso or anteiso position has the same qualitative and a similar quantitative effect in both model systems.

The results obtained in the present study confirm those obtained in earlier work. The membranes of *Bacillus subtilis* and *Staphylococcus aureus* [25], which are naturally rich in lipids containing branched-chain fatty acids, and the membranes of *Escherichia coli* artificially enriched in these fatty acids [26], have been reported to exhibit several unusual properties below their phase transition temperatures. The sharp 4.2 Å reflection usually observed in wide-angle X-ray diffraction experiments, which is associated with reflections from the closely packed hydrocarbon chains in gel state lipid, is replaced by a broader reflection with a spacing of 4.3–4.4 Å in these membranes. Moreover, the lateral aggregation of intramembranous protein particles, which is normally observed by freeze-fracture electron microscopy in membranes below their phase transition temperatures, does not occur in these systems, nor in *Acholeplasma laidlawii* B membranes when artificially enriched in branched-chain fatty acids [25,27]. Finally, pig pancreatic phospholipase A_2, which cannot hydrolyze gel-state phosphatidylglycerol in *A. laidlawii* membranes enriched with linear saturated or unsaturated fatty acids, can attack this phospholipid in membranes enriched in methyl iso- or anteiso-branched fatty acids, even at temperatures below the phase transition lower boundary temperature [28]. However, the activity of several enzymes associated with *B. subtilis* and *S. aureus* membranes [25], the rate of β-galactoside transport in *E. coli* [26] and the ATPase activity [27] and growth rate [7] in *A. laidlawii* B enriched in branched-chain fatty acids, all decline rapidly at temperatures below the phase transition midpoint, just as is observed for membranes containing linear saturated or unsaturated fatty acids [29]. Therefore, although the low-temperature lipid phases is biological as well as model membranes containing branched-chain fatty acids are somewhat expanded relative to the gel state of membranes containing non-branched fatty acids, apparently insufficient lipid orientational disorder and fluidity remain to support normal membrane function.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and the Medical Research Council of Canada. We thank Dr. Ruthven N.A.H. Lewis, Mrs. Nanette Mak and Mr. Thomas Härtner for assistance.

References

1 Kaneda, T. (1977) Microbiol. Rev. 41, 391–418
2 Rodwell, A.W. and Peterson, J.E. (1971) J. Gen. Microbiol. 68, 173–186
3 Silbert, D.F., Ladenson, R.C. and Honegger, J.L. (1973) Biochim. Biophys. Acta 311, 349–361
4 Silvius, J.R. and McElhaney, R.N. (1978) Can J. Biochem. 56, 462–469
5 Silvius, J.R. and McElhaney, R.N. (1979) Chem. Phys. Lipids 24, 287–296
6 Silvius, J.R. and McElhaney, R.N. (1980) Chem. Phys. Lipids 26, 67–77
7 McElhaney, R.N. (1974) J. Mol. Biol. 84, 145–157
8 Blume, A., Dreher, R. and Poralla, K. (1978) Biochim. Biophys. Acta 512, 489–494
9 Silvius, J.R., Mak, N. and McElhaney, R.N. (1980) Biochim. Biophys. Acta 597, 199–215
10 McElhaney, R.N. (1976) in Extreme Environments. Mechanisms of Microbial Adaptation (Heinrich, M.R., ed.), pp. 255–281, Academic Press, New York
11 Poralla, K., Kannenberg, E. and Blume, A. (1980) FEBS Lett. 113, 107–110
12 Silvius, J.R., Read, B.D. and McElhaney, R.N. (1979) Biochim. Biophys. Acta 555, 175–178
13 Blume, A. (1979) Biochim. Biophys. Acta 557, 32–44
14 Privalov, P.L., Plotnikov, V.V. and Filimonov, V.V. (1975) J. Chem. Thermodyn. 7, 41–47
15 Chapman, D., Owens, N.F., Phillips, M.C. and Walker, D.A. (1969) Biochim. Biophys. Acta 183, 458–465
16 Müller-Landau, F. and Cadenhead, D.A. (1979) Chem. Phys. Lipids 25, 315–328
17 Albrecht, O., Gruler, H. and Sackmann, E. (1981) J. Colloid Interface Sci. 79, 319–339
18 Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132
19 Rohmer, M., Bouvier, P. and Ourisson, G. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 847–851
20 Kannenberg, E., Poralla, K. and Blume, A. (1980) Naturwissenschaften 67, 458–459
21 Mabrey, A. and Sturtevant, J.M. (1978) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 9, pp. 237–274, Plenum Press, New York
22 McElhaney, R.N. (1982) Chem. Phys. Lipids 30, 229–259

23 Levin, I.R. and Bush, S.F. (1981) Biochim. Biophys. Acta 640, 760–766
24 Demel, R.A., Geurts van Kessel, W.S.M., Zwaal, R.F.A., Roelofson, B. and Van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 97–107
25 Haest, C.W.M., Verkleij, A.J., De Gier, J., Scheck, R., Ververgaert, P.H.J.T. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 356, 17–26
26 Legendre, S., Letellier, L. and Shechter, E. (1980) Biochim. Biophys. Acta 602, 491–505
27 Silvius, J.R. and McElhaney, R.N. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1255–1259
28 Bouvier, P., Op den Kamp, J.A.F. and Van Deenen, L.L.M. (1981) Arch. Biochem. Biophys. 208, 242–247
29 McElhaney, R.N. (1982) in Current Topics in Membranes and Transport (Razin, S. and Rottom, S., eds.), pp. 317–380, Academic Press, New York