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The interactions of 1-palmitoyl-2-oleylphosphatidylcholine and bovine brain cerebroside

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Model membranes composed of 1-palmitoyl-2-oleylphosphatidylcholine (POPC) and bovine brain galactocerebroside (BOV-CER) have been studied by differential scanning calorimetry (DSC). POPC is a naturally occurring phospholipid, and BOV-CER is a major component of the myelin membrane. POPC and BOV-CER are immiscible in the gel state over the composition range 0–70 mol% BOV-CER. At most POPC/BOV-CER ratios, broad dual-peaked acyl chain transitions are observed, characteristic of the co-existence of a fluid POPC-rich liquid-crystalline phase and a solid BOV-CER-rich gel phase over a wide temperature range.

Cerebroside is a monoglycosyl glycosphingolipid which is found in significant concentrations only in certain highly specialized membranes. Cerebroside makes up approximately 20% of the lipid of the myelin membrane [1], which acts as a multilamellar insulator around axons, allowing fast saltatory conduction of electrical impulses. Bovine brain cerebroside (BOV-CER) model membranes exhibit an acyl chain order-disorder transition at 67°C, which is significantly higher than mammalian body temperature [2–6]. It is likely that this physical property provides the basis for a major function of cerebroside: to provide increased order to myelin, thus decreasing permeability to ions and facilitating saltatory conduction. Cerebroside is also found in significant quantities in the intestinal brush border [7,8] and in the granular layer of the epidermis [9]; cerebroside may contribute to the impermeability of membranes in these areas.

Ruocco et al. [10] have extensively studied the interactions of synthetic palmitoyl cerebroside (C16 : 0-CER) with dipalmitoylphosphatidylcholine (DPPC), using differential scanning calorimetry (DSC) and X-ray diffraction. This work demonstrated complete miscibility of these two lipids in mixtures containing < 23 mol% C16 : 0-CER. At higher C16 : 0-CER contents, an excess cerebroside phase was observed at all temperatures below 82°C. Maggio et al. [11] have reported that mixtures of BOV-CER with DPPC exhibit gel phase immiscibility over the compositional range 0–46% BOV-CER. In both the DPPC/C16 : 0-CER and DPPC/BOV-CER systems, the calorimetric transitions at low cerebroside contents (< 25% cerebroside) occur over a relatively narrow temperature range (2–10 Cdeg) [10,11]. In the

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Abbreviations: BOV-CER, bovine brain cerebroside; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoyl-PC; POPC, 1-palmitoyl-2-oleyl-PC; C16 : 0-CER, *N*-palmitoyl cerebroside; DSC, differential scanning calorimetry.

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present study, we have investigated the interactions of the naturally occurring phospholipid 1-palmitoyl-2-oleylphosphatidylcholine (POPC) and isolated BOV-CER. This work demonstrates that POPC and BOV-CER are immiscible in the gel state and are only partially miscible at physiologically relevant temperatures at most POPC/BOV-CER ratios.

Cerebrosides were extracted from fresh bovine brains by using the procedure of Radin [12]. Alkenyl ether and ester linkages were cleaved by iodinolysis and alkaline methanolysis, respectively [12]. Cerebrosides were then isolated by chromatography on diethylaminoethylcellulose according to Rouser et al. [13] to remove sulfatides and subsequently on silicic acid. Dipalmitoylphosphatidylcholine (DPPC) was synthesized by acylation of glycerylphosphorylcholine with the acyl imidazole of palmitic acid [14]. POPC was synthesized by phospholipase A_2 digestion of DPPC and reacylation, according to Gupta et al. [15]. Lipid samples in CH_2Cl_2/CH_3OH (2:1, v/v) were dried under N_2 in Perkin-Elmer DSC sample pans (50 μl capacity), desiccated under a vacuum overnight, hydrated with distilled deionized H_2O and sealed. The lipid concentration was 14 wt%. Scanning calorimetry was carried out using a Perkin-Elmer DSC-2 scanning calorimeter.

Differential scanning calorimetry traces for aqueous dispersions of POPC, BOV-CER and 1/1 POPC/BOV-CER are presented in Fig. 1. POPC exhibits a sharp acyl chain order-disorder transition with a maximum (T_m) at $-2°C$. BOV-CER exhibits a broad transition with T_m at 67°C, as previously described [2–5]. The 1/1 POPC/BOV-CER dispersion exhibits a broad double-peaked acyl chain order-disorder transition which spans the temperature range from $-5°C$ to 63°C, with T_m values at $-1.5°C$ and 50°C. Various POPC/BOV-CER mixtures were studied by DSC, and the onset (T_o) and completion (T_c) temperatures of the order-disorder transition are plotted as a function of composition in Fig. 2. The transition maxima are also plotted. Certain features of the mixing of POPC and BOV-CER are immediately obvious. Over the composition range 0–70 mol% BOV-CER, T_o is invariant. This indicates the co-existence of two immiscible gel phases at temperatures below the T_o versus composition

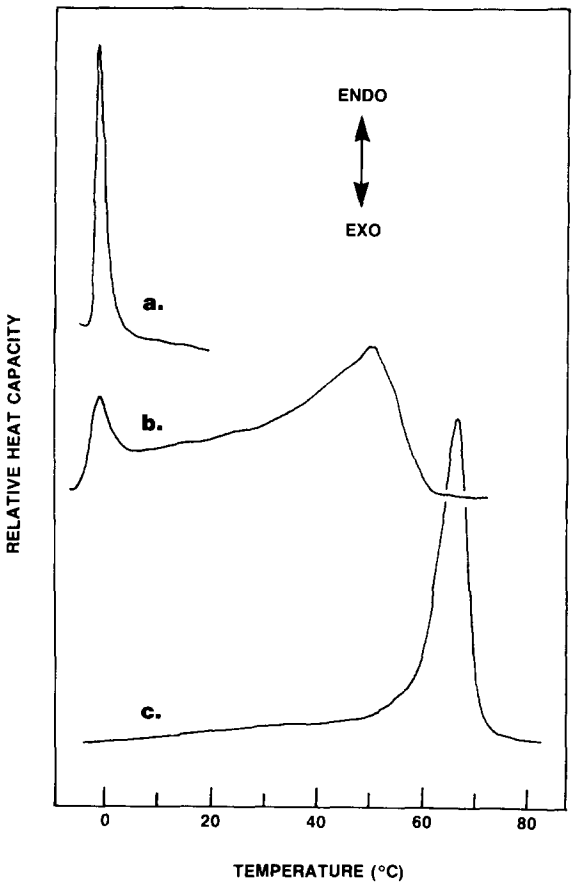

Fig. 1. Differential scanning calorimetry traces for (a) POPC, (b) 1/1 POPC/BOV-CER, and (c) BOV-CER model membranes. Heating runs at 5 Cdeg/min, after a 40 Cdeg/min cooling run which started at a temperature above the transition completion temperature and ended at $-13°C$. The calorimetric heating run was started immediately after cooling, in order to prevent freezing of supercooled excess water. Slowing the cooling rate to 5 Cdeg/min had no significant effect on the subsequent heating run.

line (solidus). Similar behavior has been previously observed for mixtures of POPC with DPPC or distearoyl-PC (DSPC) [16]. Phosphatidylcholines which differ in T_m by > 33 Cdeg generally exhibit gel phase immiscibility, based on consideration of a large number of binary phase diagrams [16]. POPC and BOV-CER differ in T_m by 69 Cdeg and would be expected to exhibit gel state immiscibility on the basis of the T_m difference alone.

The T_c versus composition line (liquidus) in Fig. 2 has no flat regions, indicating that no

liquid-crystalline immiscibility of POPC and BOV-CER occurs. However, the shape of the liquidus is indicative of some non-ideality of mixing in the liquid-crystalline state [17] and is similar to that observed for the POPC/DSPC system [16]. In the case of BOV-CER, it would be expected that intercerebroside hydrogen bonding might favor cerebroside clustering in the POPC/BOV-CER liquid-crystalline state, resulting in the observed non-ideality. In the case of POPC/DSPC, the observed nonideality [16] may result from packing disruptions caused by the *cis* double bond of the POPC oleate chain.

It is interesting to consider the behavior of POPC/BOV-CER mixtures at a physiologically relevant temperature, e.g., 37°C. At low cerebroside concentrations typical of most membranes (1–5%), BOV-CER and POPC form a mixed fluid liquid crystal. This is consistent with the studies of Sharom and Grant [18] using a spin-labeled cerebroside at low concentration. At 20% BOV-CER, the concentration found in myelin, a phase separation exists at 37°C consisting of a large amount of a fluid liquid crystalline phase containing approximately 90% POPC and 10% BOV-CER and a small amount of a gel phase containing approximately 10% POPC and 90% BOV-CER (Fig. 2). In myelin, cerebroside is likely to be asymmetrically distributed, with approximately 40% cerebroside in the extracellular monolayer of the membrane (at the external apposition or intra-period dense line) [19]. At 40% BOV-CER and 37°C, Fig. 2 again indicates the co-existence of an 90/10 POPC/BOV-CER fluid liquid crystalline phase and a 10/90 POPC/BOV-CER solid gel phase. Application of the lever rule indicates that the bilayer consists of approximately 60% fluid liquid crystalline phase and approximately 40% solid gel phase. Myelin, of course, also contains large quantities of cholesterol. If the contention is correct that the extracellular myelin monolayer contains 50% cholesterol [20], then the composition of the extracellular monolayer would be approximately 50/40/10 cholesterol/cerebroside/other polar lipids. This corresponds to 80% BOV-CER in Fig. 2, at which composition the POPC/BOV-CER bilayers are almost entirely in the gel state at 37°C. Studies of the interaction of BOV-CER with cholesterol indicate that 40–50% cholesterol eliminates the order-disorder transition of BOV-CER [2–4]. Furthermore, intact myelin and extracted myelin lipids exhibit no cooperative thermal transitions while cholesterol-depleted myelin lipids exhibit a broad cooperative phase transition [4,21]. Taken together, these studies suggest that any potential cerebroside/phospholipid phase separation in myelin at 37°C is probably eliminated by interactions with cholesterol. However, the presence of small cerebroside clusters is possible if such clusters are small enough to preclude the occurrence of cooperative acyl chain transitions.

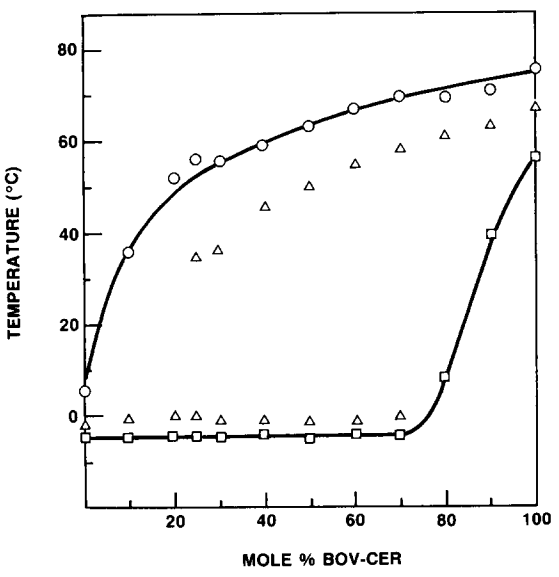

Fig. 2. Thermal behavior of POPC/BOV-CER mixed bilayers as a function of composition. Squares: transition onset temperatures, T_o. Circles: transition completion temperatures, T_c. Triangles: transition maxima, T_m.

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References

1 Nørton, W.T. (1975) Nerv. Syst. 1, 467–481
2 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 21, 303–306

3 Bunow, M.R. (1979) Biochim. Biophys. Acta 574, 542–546
4 Linington, C. and Rumsby, M.G. (1981) Neurochem. Int. 3, 211–218
5 Curatolo, W. (1982) Biochemistry 21, 1761–1764
6 Curatolo, W. and Jungalwala, F.B. (1985) Biochemistry 24, 6608–6613
7 Hauser, H., Howell, K., Dawson, R.M. and Bowyer, D.E. (1980) Biochim. Biophys. Acta 602, 567–577
8 Hansson, G.C. (1983) Biochim. Biophys. Acta 733, 295–299
9 Gray, G.M., King, I.A. and Yardley, H.J. (1978) J. Invest. Dermatol. 71, 131–135
10 Ruocco, M.J., Atkinson, D., Small, D.M., Skarjune, R.P., Oldfield, E. and Shipley, G.G. (1981) Biochemistry 20, 5957–5966
11 Maggio, B., Ariga, T., Sturtevant, J.M. and Yu, R.K. (1985) Biochim. Biophys. Acta 818, 1–12
12 Radin, N.S. (1976) J. Lipid Res. 17, 290–293
13 Rouser, G., Kritchevsky, G. and Yamamoto, A. (1976) Lipid Chromatogr, Anal. (2nd Edn.) 3, 713–776
14 Boss, W., Kelley, C. and Landsberger, F. (1975) Anal. Biochem. 64, 289–292
15 Gupta, C.M., Radhakrishnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4315–4319
16 Curatolo, W., Sears, B. and Neuringer, L.J. (1985) Biochim. Biophys. Acta 817, 261–270
17 Lee, A.G. (1978) Biochim. Biophys. Acta 507, 433–444
18 Sharom, F.J. and Grant, C.W. (1975) Biochem. Biophys. Res. Commun. 67, 1501–1506
19 Linington, C. and Rumsby, M.G. (1980) J. Neurochem. 35, 983–992
20 Caspar, D.L.D. and Kirschner, D.A. (1971) Nature, New Biol. 231, 46–52
21 Ladbrooke, B.D., Jenkinson, T.J., Kamat, V.B. and Chapman, D. (1968) Biochim. Biophys. Acta 164, 101–109