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Phosphatidylcholine-Cerebrosides Interactions: Influence of Cerebroside Acyl Chain Character†

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We have measured the swelling properties, and force decay with bilayer separation, for single phase multi-lamellar arrays made from dioleoylphosphatidylcholine, and cerebrosides with primarily non-hydroxl acyl chains. The phase characteristics and interactive forces are found to be similar to bilayers made from DOPC and cerebrosides with primarily hydroxyl acyl chains. In all cases, cerebrosides are found to have little effect on the hydration and electrostatic forces.

INTRODUCTION

Membrane components with glycosylated segments located at the bilayer-solvent interface are thought to be involved in both the long range and short range communication between cells.[1-2] At long ranges, bilayer separations greater than 100 Å, membrane-membrane interactions can be influenced by the van der Waals attractive force[3] and the electrostatic repulsive force,[4] which are both non-specific forces. At short ranges, bilayer separations less than 30 Å, the hydration repulsive force[5] must be overcome by specific interactions between moieties at the membrane-solvent interface. It has been theoretically predicted that the presence of glycosylated segments at the bilayer interface in the form of a "fuzzy layer" can influence the van der

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Waals attractive force.[6-7] A simple modelling of this interaction used aqueous media containing the sugars sucrose or glucose between phosphatidylcholine bilayers.[8] A decrease in bilayer separation is observed as the sugar concentration increases indicating an increase in the van der Waals attractive force.

We have modelled the effect of a "fuzzy layer" using a neutral glycolipid, cerebrosides with primarily hydroxy acyl chains (HAC) in dioleoylphosphatidylcholine bilayers, which forms a single lamellar phase in all solvents examined.[9] The forces between bilayers were measured using osmotic pressure.[10] Dextran was added to the aqueous media containing the lipid multi-lamellar arrays, and applies a compressive force on the bilayers due to competition between the hydration requirement of the lipid head groups and the dextran for the available water. DOPC/cerebrosides swelled in an electrostatic fashion in the presence of 30mM $CaCl_2$ if the bilayers were in the liquid crystalline phase. The DOPC hydration and electrostatic forces apparently remained unchanged with the addition of cerebrosides while the van der Waals force qualitatively increased with increasing cerebroside content in the bilayer. We can conclude that the glycosylated moieties dissolve in the aqueous compartment, and that cerebrosides act as a "filler" between DOPC molecules in the bilayers.

In this report we examine the influence of changing the acyl chain character of the cerebrosides from primarily hydroxy acyl chain (HAC) to non-hydroxy acyl chains (NAC) on the forces between DOPC/cerebroside bilayers in 30mM $CaCl_2$. Although there have been a number of studies on cerebroside-phospholipid interactions,[11-21] few[13,15] have studied the consequence of cerebroside acyl chain character. Deuterium NMR studies[15] indicate that the cerebroside acyl chain character effects the disorder of the phospholipid head group motion, while DSC studies[13] indicate different hydration requirements for cerebroside bilayers with different acyl chain species. Our gravimetric X-ray results indicate the DOPC/cerebrosides bilayers swell to approximately the same extent regardless of the cerebrosides acyl chain species, while our force measurements indicate that the forces at the bilayer interface are unchanged. Although the acyl chain character of the cerebrosides may affect the packing within the phospholipid bilayer, the interactions between bilayers remain unchanged.

MATERIALS AND METHODS

Dioleoylphosphatidylcholine and Type II bovine brain cerebrosides (higher spot, containing primarily the non-hydroxy form of the fatty

acids) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used without further purification. Lipids were mixed by dissoving in chloroform at approximately 30°C. The details are presented elsewhere.[9] All salts were of reagent grade and were diluted to the desired molarity with singly distilled water.

Gravimetric samples were prepared by adding a known mass of solute to a known mass of lipid. Samples used for force measurements were prepared by adding the lipid to solute containing a known weight percent Dextran T2000 (Pharmacia Fine Chemicals, Piscataway, N.J. USA). The samples were then allowed to equilibrate at room temperature for 36-48 hours in hermetically sealed bottles. Samples allowed to equilibrate longer than this time showed no change in structure, indicating equilibrium had been reached.

The resultant lipid samples were mounted in X-ray sample holders between mica sheets. Powdered teflon was placed in the sample area to serve as an X-ray diffraction standard. Samples mounted in this fashion were stored at 0°C until subjected to X-ray diffraction.

X-ray diffraction of each such sample was performed using a General Electric XRD-4 generator with a copper anode Ca/8L tube. The copped K_α-line was isolated by use of a half-millimeter Nickel foil as a filter. Modified Debye-Scherrer cameras were used containing an evacuated chamber between the sample and film. X-ray diffraction patterns were obtained at room temperature. Low angle diffraction lines were measured using the Bragg relation and compared to the 4.87 Å repeat of teflon. The number of sets of equally spaced diffraction lines yields the number of phases present in the lamellar system that are resolvable by X-ray diffraction.

From the X-ray diffraction determination of the lamellar repeat distance d; the bilayer thickness d_l, the water thickness d_w may be inferred from the volume fraction, Φ, of lipid in the suspension. Using the standard assumptions that 1) the fatty acyl chains of the lipids do not overlap across the bilayer to any great extent, 2) the mixed lipid system is miscible, 3) water is excluded from the hydro carbon regions of the bilayer, and 4) once the lipid bilayers stop swelling, any extra water added to the system forms a separate phase, then the above quantities may be calculated from the following expressions:

$$d_l = \Phi d \tag{1}$$

$$d_w = d - d_l \tag{2}$$

$$\phi = \frac{1}{1 + \dfrac{(1 - c)v_w(1 + R)}{c(Rv_c + v_l)}} \tag{3}$$

$$\text{where } R = (Mw_c/Mw_l)([\text{Cerebrosides}]/[\text{PC}]). \tag{4}$$

The partial specific volumes of the DOPC, cerebrosides, and water are represented by v_l, v_c and v_w. The weight fraction of the lipid mixture in the suspension is c. Mw_c and Mw_l are the respective molecular weights of the cerebrosides and DOPC, and [cerebrosides] and [PC] are the molar concentrations of cerebrosides and DOPC. We have assumed the following values for these quantities: Mw_c = 840 g/mole, Mw_l = 789 g/mole, v_w = 1.002 cm^3/g, v_l = 0.9884 cm^3/g (see Ref. 22), and v_c = 1.02 cm^3/g (see Ref. 18).

The details of the force measurements have been described elsewhere.[10,23] The repeat spacings obtained from this technique are compared to those obtained gravimetrically to determine d_l and d_w.

The magnitude of the electrostatic force at any bilayer separation may then be found experimentally from the relation

$$F_{es} = \Pi + F_{vdw} - F_{hyd}.$$

where F_{vdw} is the attractive van der Waals force and F_{hyd} is the repulsive hydration force. The non-linear Poisson-Boltzmann differential equation governing the electrostatic potential between bilayers has been solved by Lis, et al.[24] Thus, by equating the measured electrostatic force at a given bilayer separation to the derived electrostatic force, one may calculate the electrostatic potential at the bilayer surface, and by applying Gauss' Law one may equate the slope of the potential evaluated at the bilayer to the surface charge density on the bilayer surface. The electrostatic force was analyzed in 30mm $CaCl_2$ to allow direct comparison to previous studies on phosphatidylcholines.[24]

The applied osmotic pressure of the dextran is determined from the known weight percent dextran added to the lipid mixtures and the corresponding force calibration curve presented by LeNeveu, et al.[23]

RESULTS AND DISCUSSION

The DOPC/cerebroside mixtures studied produced only single phase lamellar X-ray diffraction patterns. A molar ratio of 1 DOPC/1 cer-

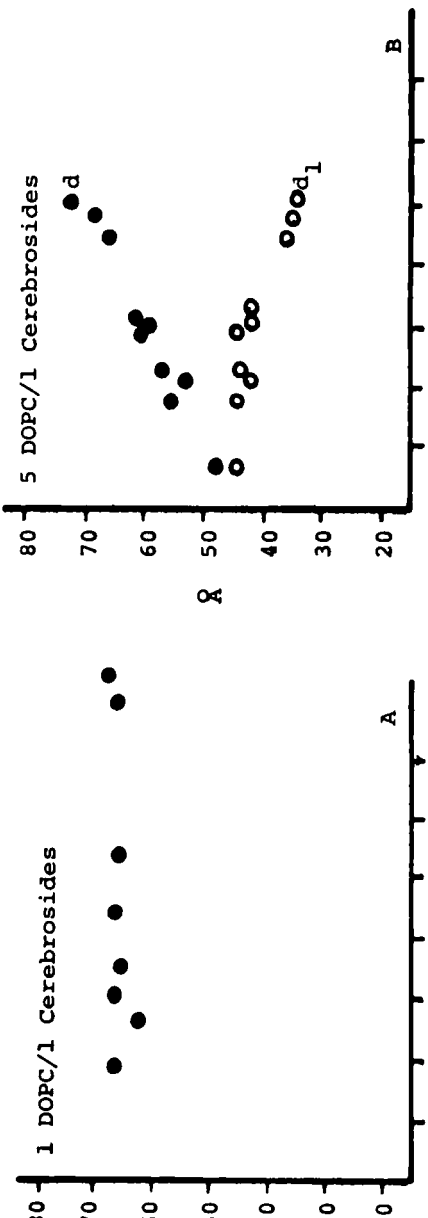

FIGURE 1 Bilayer repeat, d, and bilayer thickness, d_1, as a function of lipid concentration for several DOPC/cerebroside (NAC) mixtures at 23°C. The volume fraction of the lipid, Φ is approximated by the weight percent lipid.

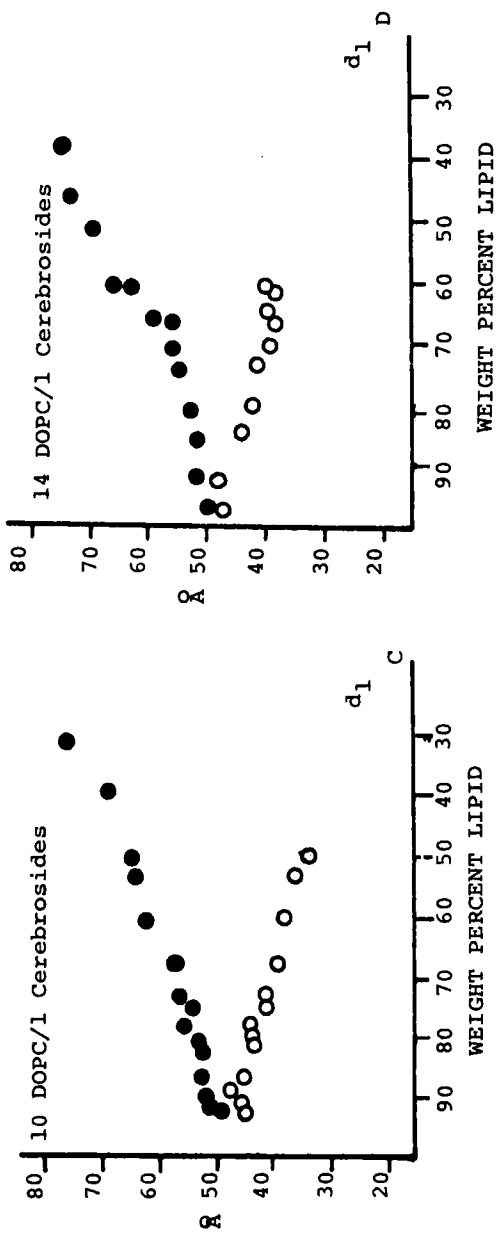

FIGURE 1 (continued)

ebroside in 30 mM $CaCl_2$ exhibited limited swelling (Figure 1A) and the high angle diffraction line at 4.2 Å indicated that the mixture was in the gel phase. The lower cerebrosides content mixtures (5/1, 10/1, 14/1) exhibited unlimited swelling in the presence of 30 mM $CaCl_2$ (Figures 1B, C, D). The high angle X-ray diffraction lines for these mixtures indicated that the bilayers were in the liquid crystalline phase. The swelling behavior for DOPC/cerebroside mixtures with either the hydroxy or the non-hydroxy acyl chains is very similar.[9] Both sets of mixtures show unlimited swelling characteristic of DOPC in 30 mM $CaCl_2$[24] only after the gel-liquid crystalline phase transition.

The mixtures which showed swelling characteristic of charged DOPC bilayers[24] were examined using the osmotic pressure technique. Data for the 10/1 and 14/1 mixtures are shown in Figure 2. The bilayers for both mixtures swell similarly which is similar to DOPC in 30 mM $CaCl_2$ for the hydration and electrostatic force decays respectively. The least squares fit lines in the hydration and electrostatic regions are expressed as exponential functions. The decay equations for the hydration force for the two mixtures (Table I) are not statistically

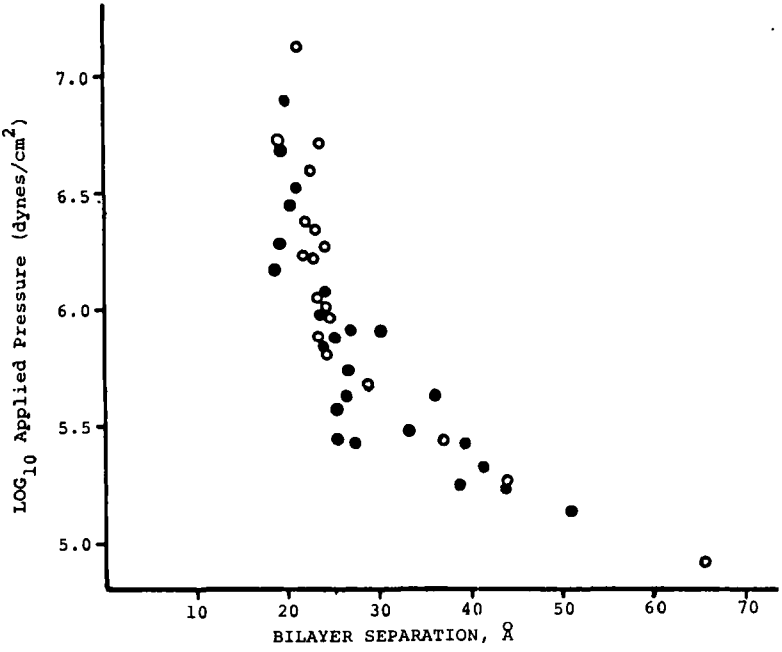

FIGURE 2 Applied osmotic pressure versus bilayer separation for
· 10 DOPC/1 cerebrosides (NAC)
· 14 DOPC/1 cerebrosides (NAC)

different from that found for DOPC in H_2O (see Ref. 25), (i.e. $P = 10^{9.6} \exp(-d_w/2.9\ \text{Å})$ dynes/cm^2). The electrostatic force decays can also be fitted to a least squares exponential function. Once again the electro-static force decay (Table II) remains statistically the same for DOPC bilayers with and without cerebrosides (NAC). The lack of cerebroside effect on the electrostatic force for DOPC bilayers indicates that cerebrosides act as an ideal filler molecule in the bilayer. This is directly contrary to the effects seen with cholesterol. Cholesterol causes a decrease in the swelling behavior of DPPC bilayers in 30 mM $CaCl_2$ (Lis, et al., to be published). Cholesterol thus helps to regulate the interaction between bilayers while cerebrosides are passive.

The force decay for 14/1, DOPC/cerebrosides, mixtures with cerebroside molecules with hydroxy acyl chains or non-hydroxy acyl chains are statistically similar (Figure 3). This result must be interpreted in light of the findings for different packing structures within phospholipid bilayers for cerebrosides with hydroxy acyl chains versus those with non-hydroxy acyl chains.[15] The interactive forces are a function of the bulk structure of the bilayer and solvent. Since the same concentration of sugar molecules is in the solvent for both mixtures, the hydration force remains unchanged. Thus differences in cerebrosides acyl chain species may be needed to change the packing for compressibility within the bilayer, they are not sufficient for

TABLE I

Least Squares Hydration Force Decay Lines for 10/1 and 14/1 DOPC/cerebrosides

10/1(NAC)P_{hyd}	=	$10^{(8.7\pm0.6)}\exp[-d_w/(3.8\pm0.9)\text{Å}]$ dyne/cm^2
14/1(NAC)P_{hyd}	=	$10^{(9.7\pm1.3)}\exp[-d_w/(3.0\pm1.1)\text{Å}]$ dyne/cm^2
14/1(HAC)P_{hyd}	=	$10^{(8.4\pm0.3)}\exp[-d_w/(4.2\pm0.7)\text{Å}]$ dyne/cm^2

TABLE II

Least Squares Electrostatic Force Decay Lines for 10/1 and 14/1 DOPC/cerebrosides

10/1(NAC)P_{el}	=	$10^{(6.1\pm0.2)}\exp[-d_w/(23\pm6)\text{Å}]$ dyne/cm^2
14/1(NAC)P_{el}	=	$10^{(6.4\pm0.1)}\exp[-d_w/(19\pm1)\text{Å}]$ dyne/cm^2
14/1(HAC)P_{el}	=	$10^{(6.4\pm0.1)}\exp[-d_w/(16\pm2)\text{Å}]$ dyne/cm^2

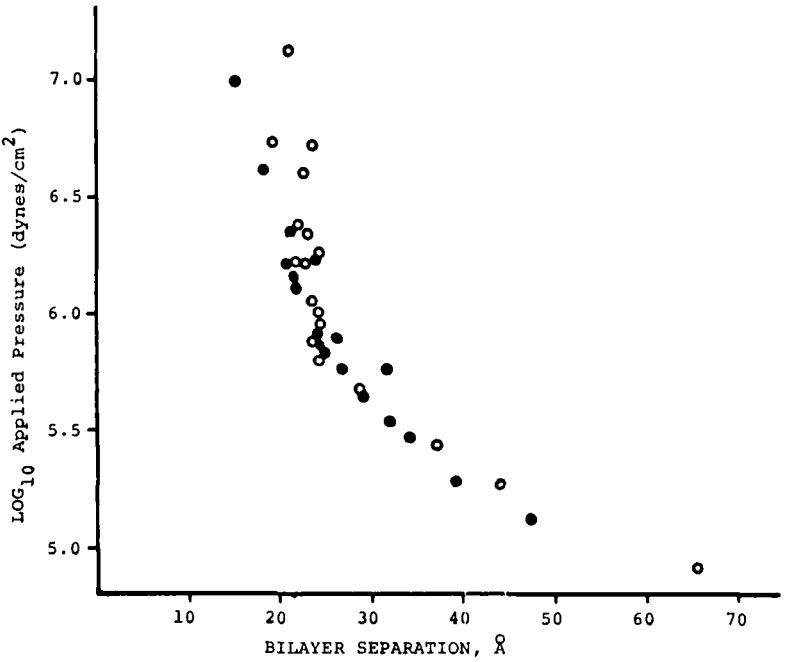

FIGURE 3 Applied osmotic pressure versus bilayer separation for 14 DOPC/1 cerebrosides:
· hydroxy acyl chains (NAC)
· non-hydroxy acyl chains (NAC)

changing the interactive forces. That the force decay for liquid crystal and gel state phosphatidylcholine are similar is consistent with previous findings.[25]

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