Phase Stability of Phosphatidylcholines in Dimethylsulfoxide Solutions

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ABSTRACT The temperature dependence of the phase stability of dispersions of dimyristoyl, dipalmitoyl, and distearoyl derivatives of phosphatidylcholines in excess aqueous dimethylsulfoxide has been examined by differential scanning calorimetry and synchrotron x-ray diffraction methods. There was a close correlation between the enthalpic transitions and the structural changes associated with the pre- and main transitions of the phospholipids in the range of concentrations up to mole fractions of dimethylsulfoxide in water of 0.1333. The temperatures of the pre- and main transitions of the three phospholipids were found to increase linearly with increasing mole fraction of dimethylsulfoxide. The difference in phase stability between the lamellar gel and ripple phases induced by increasing dimethylsulfoxide concentration resulted in disappearance of the ripple phase and direct transition between lamellar gel and lamellar liquid-crystal phases. The effect of changing the properties of the solvent by the addition of dimethylsulfoxide on the dimensions of dipalmitoylphosphatidylcholine and solvent layers of the bilayer repeat structure has been determined from electron density distribution calculations. The lamellar repeat spacing recorded at 25°C decreased from 6.36 nm in aqueous dispersion to 6.04 nm in a dispersion containing a mole fraction of 0.1105 dimethylsulfoxide. The results indicate that dipole interactions between solvent and phospholipid and dielectric properties of the solvent are important factors in the determination of the structure of saturated phosphatidylcholines.

INTRODUCTION

Phosphatidylcholines are among the most ubiquitous membrane lipid class and represent the major bilayer-forming lipid in most biological membranes. They are known to form a number of different lamellar phases in water, including two crystal phases, two gel phases, and a liquid-crystal lamellar phase (Lewis et al., 1987; Tenchov, 1991; Koynova et al., 1995). The structure of these different phases and the temperature at which transitions take place between them depend on the amphipathic properties of the molecules. There is current experimental and theoretical interest in the mechanism and driving forces that govern thermotropic transitions in phosphatidylcholine-water mixtures.

Recent attention has been focused on transitions between lamellar crystal phases in saturated phosphatidylcholines (Koynova et al., 1995) and in transitions between gel phases and the lamellar liquid-crystal phase (Tenchov et al., 1989). The present study addresses the forces and structural rearrangements associated with the latter transitions in saturated phosphatidylcholines. It was originally thought that the transition between the gel and ripple phases, the so-called pre-transition, was driven by a rearrangement of the polar group of the phospholipid (Chapman, 1973), but it was subsequently shown that the pre-transition involves a considerable increase in the rotational motion of the hydrocarbon chains (Marsh, 1980). The precise structure of the ripple phase is still conjectural, with some models suggesting the coexistence of fluid-like and solid-like micro-domains of the hydrocarbon region of the bilayer (Wittebort et al., 1981; Schneider et al., 1983; Tsuchida and Hatta, 1988), whereas others account for the structural deformations by domain differences in packing of the polar group (Meyer et al., 1994). The force driving the thermotropic transition from gel to ripple phase is said to be the interfacial tendency for lateral expansion (the head-water-head repulsion) within the bilayer assembly (Cevc, 1991a). Water is a key element, and factors that influence water structure or "solvent" characteristics will affect the transition.

Modulation of the ripple phase stability by small solute molecules present in the water layer can provide useful information about the mechanism of the ripple phase formation. Light scattering (Veiro et al., 1987) and x-ray diffraction and calorimetric methods (Tamura-Lis et al., 1990) have shown that short-chain alcohols decrease the pre-transition temperature of dipalmitoylphosphatidylcholine. Solutes like proline (Tsvetkova et al., 1991), sugar molecules, and inorganic salts (Cevc, 1991a) are also known to cause destabilization of the ripple phase. We have extended this approach by undertaking a detailed examination of the effect of dimethylsulfoxide (DMSO), an extensively used co-solvent in cell biology (Yu and Quinn, 1994), on the structure and thermal stability of the pre- and main transition of several saturated phosphatidylcholines. Electron density distributions within the lamellar repeat of gel phases of multibilayer liposomes dispersed in aqueous DMSO have been calculated, and an analysis of attractive and repulsive forces has been performed.

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Abbreviations used: DMPC, DPPC, and DSPC, 1,2-dimyristoyl-, 1,2-dipalmitoyl-, 1,2-distearoyl-phosphatidylcholine; DMSO, dimethylsulfoxide; $L_{\beta'}$, lamellar-gel phase; $P_{\beta'}$, rippled gel phase; L_{α}, liquid-crystal phase.

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MATERIALS AND METHODS

Sample preparation

Synthetic dimyristoyl-, dipalmitoyl-, and distearoyl-phosphatidylcholine (DMPC, DPPC, and DSPC) were purchased from Avanti (Birmingham, AL) and were used without further purification. Dimethylsulfoxide (DMSO) was purchased from Sigma Chemical Co. (St. Louis, MO). Lipid dispersions with a lipid/solvent ratio of 1:2 (wt/wt) were prepared in aqueous DMSO solutions and were used to study thermotropic phase behavior. Dispersions used to determine x-ray diffraction parameters contained various lipid/solvent ratios with a mole fraction of DMSO of 0.1105. Homogeneous dispersions were obtained by subjecting the samples to several freeze-thaw cycles between about −20°C and above the respective main phase transition temperatures, interspersed with extensive vortex mixing.

Calorimetry

Differential scanning calorimetry (DSC) experiments were performed using a Perkin-Elmer DSC-2 calorimeter. Lipid dispersions in aqueous DMSO solutions (approx. 5 mg) were sealed in aluminium pans for thermal studies. Heating and cooling scans at 5°/min were recorded within the temperature ranges of 5–30°C for DMPC, 20–60°C for DPPC, and 35–70°C for DSPC. Thermal studies were also performed at a scanning rate of 2.5°/min with no significant effects on the temperature or enthalpy of respective phase transitions. The temperature of pre-transition was assigned as its peak temperature and that of main transition was determined by its onset temperature in the present study.

X-ray diffraction

Synchrotron x-ray diffraction measurements were carried out at station 8.2 of the Synchrotron Radiation Source at the Daresbury Laboratory, UK. The combination of a quadrant detector and an INEL detector was used to obtain a simultaneous record of both small-angle (~1°) and wide-angle (~20°) diffraction patterns (Bras et al., 1993). Changes in x-ray scattering intensity profiles were recorded during heating and cooling scans performed in a manner identical to that of the calorimetric measurements (Cunningham et al., 1994).

Electron density distributions across the unit cell of the lamellar repeat of DPPC dispersed in water and water-DMSO solution (x = 0.1105) were calculated as follows. Integrated intensities $I(h)$ for a range of diffraction orders (h) were obtained from low-angle scattering intensity profiles. Electron density profiles in a one-dimension space x, in arbitrary units, were then expressed as (McIntosh et al., 1989)

$$p(x) = \sum g(h)|F(h)|\cos(2\pi hx/d) \qquad (1)$$

where $F(h)$ is the structure factor and equals the root of $I(h)$ in amplitude, $g(h)$ is the phase of $F(h)$ of the hth order diffraction, and d is the repeat spacing of the multibilayers.

To calculate a true electron density profile it is first necessary to solve the phase problem. One way to approach this problem is to adopt a model-building method (Warren, 1987). In the camera configuration used in this study, five orders of diffraction were collected from the dispersion of DPPC in water and four orders from DPPC in DMSO solution (x = 0.1105). If we assume that the multilamellar structure of the dispersions are centrosymmetric, the phase angles of the structure factor $F(h)$ are restricted to either π or 0. This implies that g could only be −1 or 1 (McDaniel et al., 1983). Thus there are 32 and 16 combinations of phases for the dispersion of DPPC in water and aqueous DMSO solution, respectively, in their electron density analysis. Examination of all possible phase sets indicates that plausible combinations of phases consistent with a bilayer repeat are − − + − − for DPPC in water and − − + − for DPPC in water-DMSO solution. The result obtained for DPPC in water is in agreement with that published elsewhere for this system (Torbet and Wilkins, 1976; Quinn et al., 1995).

A more direct way to solve the phase problem is to obtain data from a swelling series in which lipid is dispersed in increasing amounts of solvent, assuming the electron density profile of the structure remains constant over the range of solvent/lipid ratios examined. Structure factors of a series of DPPC dispersions, normalized according to the procedure of Franks and Levine (1981), are plotted in Fig. 1 *A*. By reverting alternate cones in Fig. 1 *A*, all of the data points are seen to fall on a smooth curve. As structure factors in the same cone have the same phase, the phases of the transform turn out to be either − − + − or + + − +. Because the electron density in the center of the hydrocarbon regions is lower than that of the solvent layer (McDaniel et al., 1983), the former solution is taken to be correct.

Electron density profiles of the dispersions of DPPC in the swelling series calculated using this phase set are plotted in Fig. 1 *B*. It can be seen from the series of curves that there is no significant change in the dimensions of the lipid bilayer as the lipid/solvent ratio varies. The increase of the amount of aqueous DMSO solution (x = 0.1105), as expected, results in only an expansion of the solvent layer.

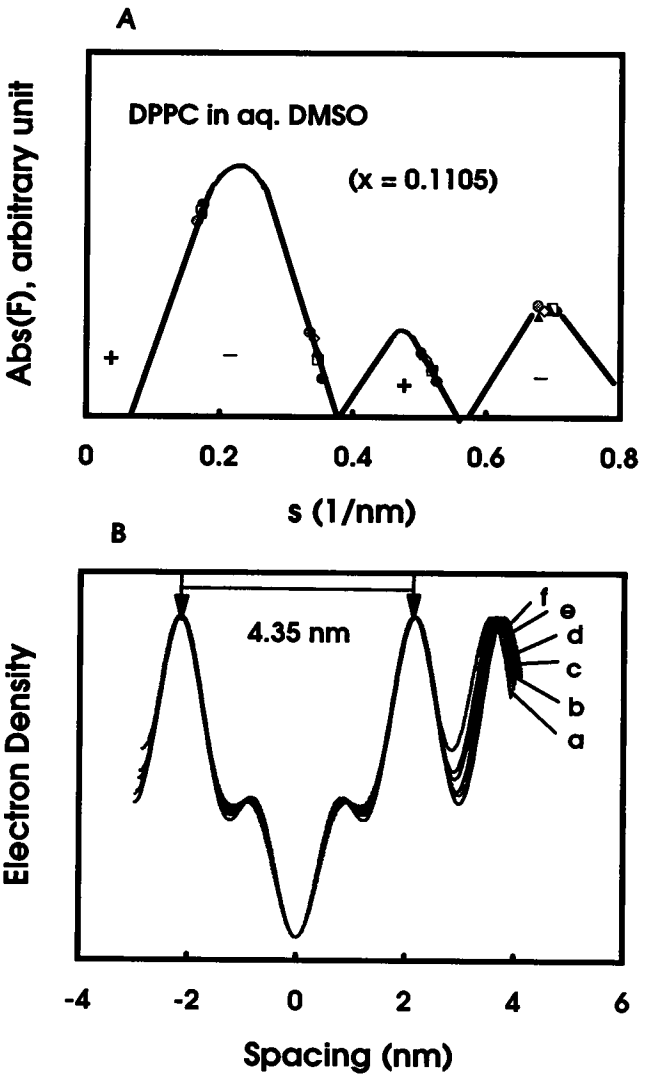

FIGURE 1 (*A*) Amplitudes of structure factor in reciprocal spacing, a swelling series of DPPC dispersed in aqueous DMSO solutions (mole fraction x = 0.1105). (*B*) Electron density profiles of DPPC dispersed in different amount of aqueous DMSO solutions with a constant mole fraction of x = 0.1105. The weight percentages of the solvent in the dispersion are: a, 18.3%; b, 32.3%; c, 35.27%; d, 41.09%; e, 46.63%; f, 49.88%.

RESULTS

Differential scanning calorimetry

The phase behavior of dispersions of DMPC, DPPC, and DSPC in aqueous DMSO solutions was examined by DSC using a scanning rate of 5°/min. Temperatures of the pre- and main transitions during heating and cooling of aqueous dispersions of phospholipids containing different mole fractions of DMSO are listed in Table 1. The values obtained for transition temperatures of these lipids in the absence of DMSO are in good agreement with published data (Lewis et al., 1987). Plots of these data in Fig. 2 show clearly that the temperature of the pre- and main transition increase with increasing mole fraction of DMSO. The relationship is preserved in both the heating (Fig. 2 *A*) and cooling (Fig. 2 *B*) modes, but there is a marked difference in temperature hysteresis between the pre- and the main transitions for each of the lipids. Thus the temperature of the main transitions differs by only 1–2°, but that of the pre-transition on cooling is about 10° lower than that of the corresponding transitions observed during heating. The hysteresis effect was reflected in the concentration of DMSO at which the pre-transition merges with the main transition. Values of the mole fraction of DMSO at which the pre-transition disappears during heating scans were 0.07, 0.04, and 0.01, and during cooling scans were 0.11, 0.10, and 0.06 for DMPC, DPPC, and DSPC, respectively.

By contrast with the effect of DMSO on transition temperature, there were no significant effects on the enthalpy of the pre- and main transitions. These values were in close agreement with published values for the respective transitions of these phospholipids in pure aqueous dispersions

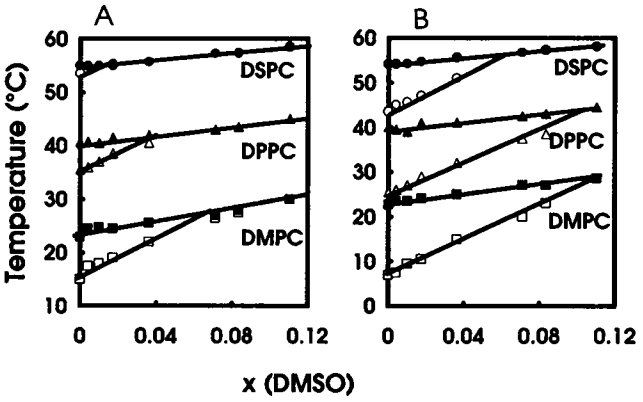

FIGURE 2 The effect of DMSO on pretransition (open symbols) and main transition (closed symbols) temperature of dimyristoyl (DMPC), dipalmitoyl (DPPC), and distearoyl (DSPC) derivatives of phosphatidylcholine dispersed in excess aqueous DMSO mixtures during heating (*A*) and cooling (*B*) at 5°/min.

(Lewis et al., 1987). Furthermore, the cooperativity of the transitions, as judged by the temperature range over which the change in specific heat capacity occurs, were not significantly affected by the presence of DMSO.

Synchrotron x-ray diffraction

Real-time x-ray diffraction patterns were recorded during heating and cooling of the aqueous dispersions of DPPC containing varying mole fractions of DMSO to correlate the thermal transitions observed by calorimetry with structural transitions in the dispersions. Low-angle and wide-angle

TABLE 1 Pre- and main transition temperatures of phosphatidylcholines in excess aqueous DMSO solutions upon heating and cooling with DSC scanning rate of 5°/min

x	0	0.0041	0.0100	0.0173	0.0362	0.0712	0.0833	0.1105
Dimyristoylphosphatidylcholine								
Heating								
T_p	15.0	17.5	18.0	19.0	22.0	26.5	27.5	—
T_m	23.0	24.5	24.7	24.5	25.5	27.0	28.0	30.0
Cooling								
T_m	22.7	23.5	23.5	24.0	25.0	27.0	27.0	28.5
T_p	7.0	7.5	9.5	10.5	15.0	20.0	23.0	—
Dipalmytoylphosphatidylcholine								
Heating								
T_p	35.5	36.0	37.0	38.5	40.5	—	—	—
T_m	40.7	40.8	40.5	41.5	42.0	43.0	43.5	45.0
Cooling								
T_m	40.0	39.5	39.0	40.9	41.0	42.5	43.0	44.5
T_p	25.5	26.0	27.0	29.0	32.0	37.5	38.5	—
Distearoylphosphatidylcholine								
Heating								
T_p	53.5	54.0	55.0	—	—	—	—	—
T_m	55.0	54.9	55.0	55.3	55.7	57.2	57.3	58.5
Cooling								
T_m	54.1	54.1	54.2	54.6	55.6	56.7	57.2	58.0
T_p	43.5	45.0	45.5	47.0	51.0	—	—	—

x, mole fraction of dimethylsulfoxide in its aqueous solution; T_p and T_m, pre- and main transition temperatures (°C); —, no transition observed.

diffraction intensities from DPPC dispersed in water and aqueous DMSO with mole fractions of 0.0362, 0.0712, and 0.1333 are shown in Fig. 3 *B*, *C*, and *D*, respectively. Only the first-order lamellar repeat spacing is shown, but up to five orders of reflection confirmed that all phases are lamellar. The ripple phase, $P_{\beta'}$, of the DPPC dispersions is typified by a low-intensity diffraction pattern of the type reported previously (Matuoka et al., 1990; Wolf et al., 1992). The gel phases ($L_{\beta'}$ and $P_{\beta'}$) were distinguished from the liquid-crystal phase (L_α) by the presence of a sharp wide-angle diffraction band centered at a spacing corresponding to about 0.42 nm, compared with a broad diffraction band at 0.46 nm.

It can be clearly seen in Fig. 3 that increasing amounts of DMSO in the aqueous medium reduce the range of temperature over which the ripple phase appears, until eventually there is a direct transition from $L_{\beta'}$ to L_α (Fig. 3 *D*). It is noteworthy that the existence of a ripple phase over a temperature range of about 1° is observed in DPPC dispersed in aqueous DMSO at a mole fraction of 0.0712 (Fig. 3 *C*); this phase was not detected by calorimetry performed under identical conditions.

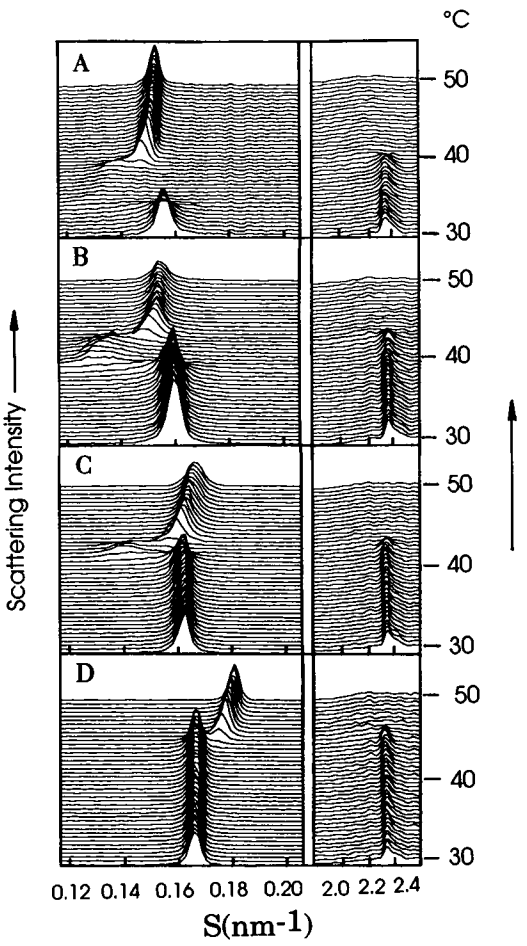

FIGURE 3 Synchrotron x-ray scattering intensity profiles recorded at low-angle (left) and wide-angle (right) from DPPC dispersed in aqueous DMSO solutions containing (*A*) 0.0; (*B*) 0.0362; (*C*) 0.0712; and (*D*) 0.1333 of DMSO during heating from 30 to 50°C at 5°/min. Each profile represents x-ray scattering intensity accumulated over 6 s.

Detailed examination of the wide-angle diffraction patterns shown in Fig. 3 reveals two major differences between $L_{\beta'}$ and $P_{\beta'}$. First, the diffraction peak from $P_{\beta'}$ phase tends to be more symmetric than that from $L_{\beta'}$ phase, consistent with previous reports (Brady and Fein, 1977). Second, there is a decrease in the wide-angle diffraction intensity during the pre-transition. Electron spin resonance studies have shown that the rotational motion of acyl chains in $P_{\beta'}$ is faster than that in the $L_{\beta'}$ phase, a consequence that requires slightly greater disorder in the chain packing (Marsh, 1980).

Another feature seen in the synchrotron x-ray diffraction data shown in Fig. 3 is the progressive shift of the small-angle diffraction pattern toward higher angles with increasing DMSO concentration. This is illustrated in Fig. 4, which shows the relationship between lamellar repeat spacings and temperature of dispersions of DPPC in different aqueous DMSO solutions. The presence of increasing amounts of DMSO caused a reduction in repeat spacings of all three mesophases. With the higher proportions of DMSO examined there was also a marked decrease in lamellar repeat spacing of the liquid-crystal phase with increasing temperature. These changes in d-spacings may be due to either a decrease in the thickness of the lipid bilayer or a reduction in dimensions of the solvent layer, or both.

To distinguish the origin of the changes in dimensions of the mesophases in the presence of DMSO, electron density profiles of DPPC in excess water and aqueous DMSO solution (x = 0.1105) at 25°C were derived. The results are shown in Fig. 5 *A*. The peak-to-peak distance d_l, taken to be a measure of the thickness of the lipid bilayer, is 4.13 nm for the aqueous dispersion of DPPC. Despite a decrease in lamellar repeat spacing of the dispersion in the presence of DMSO (Fig. 4), the apparent lipid bilayer thickness, d_l, increases to 4.35 nm. The increase in d_l could result from a decrease in the tilting angle of the acyl chains as rationalized in previous studies where such effects were observed

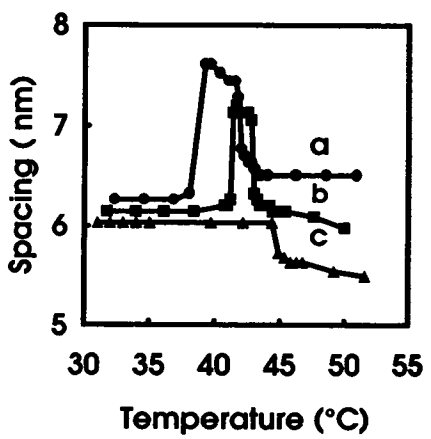

FIGURE 4 Lamellar repeat spacings vs. temperature of DPPC in aqueous DMSO solutions. Mole fractions of DMSO are (*a*) 0.0362; (*b*) 0.0712; (*c*) 0.1333.

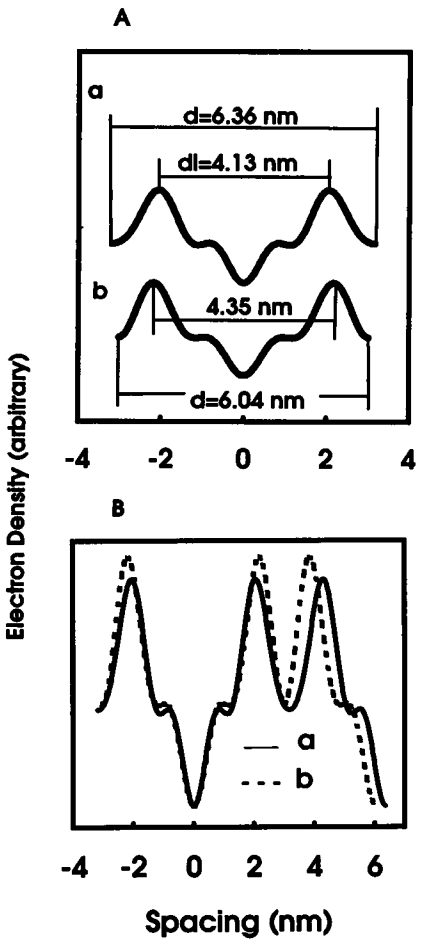

FIGURE 5 (*A*) Electron density profiles of DPPC dispersed in excess water and aqueous DMSO solutions (x = 0.1105). d, the repeat spacing of the multilamellar structure of the dispersion. (*B*) superposition of electron density profiles shown in (*A*).

(McIntosh, 1980; Quinn et al., 1995). Tilting of the acyl chains in the $L_{\beta'}$ phase of DPPC in water occurs at an angle of about 27° (Tristram-Nagle et al., 1993). The wide-angle x-ray scattering intensity profile recorded from this phase is characterized by a sharp peak centered at a spacing of 0.42 nm and a broad shoulder on the high-angle side, which arises from a packing of the tilted chains in a quasi-hexagonal lattice (Tardieu et al., 1973; Brady and Fein, 1977). To determine whether there was any change in the angle of tilt of the hydrocarbon chains that would account for the apparent increased thickness of the bilayer in the presence of DMSO, the profiles of the wide-angle scattering bands were compared and the results are presented in Fig. 6. This shows that the two profiles are almost identical for bilayers of DPPC in water and aqueous DMSO (x = 0.1105), suggesting that any change in the tilting angle is small.

An alternative explanation is that DMSO molecules localize at the lipid interface because of specific interactions with the lipid polar group. In this case the scattering from the relatively electron-dense sulfur atoms of the DMSO would extend the scattering of the phosphate group of the

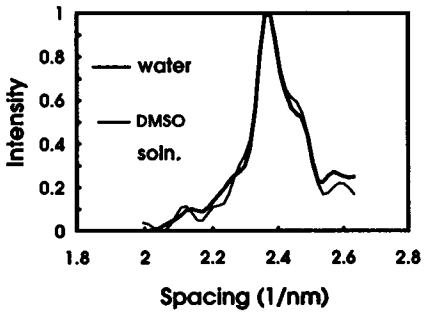

FIGURE 6 Relative synchrotron x-ray scattering intensity profiles of the wide-angle band in dispersions of DPPC in water (thick line) and aqueous DMSO (x = 0.1105) (thin line) recorded at 25°C.

phospholipid, thereby contributing to an increase in distance between the peaks of high electron density on either side of the bilayer. This was illustrated in the superposition of the two electron density profiles in Fig. 5 *B*. Direct interactions between DMSO and the polar groups of phospholipids have been suggested by Anchordoguy et al. (1991).

DISCUSSION

The present results show that the addition of DMSO to dispersions of phosphatidylcholines results in an upward shift in temperature of both the pre- and main transitions and the ultimate merger of the two transitions at sufficiently high DMSO concentrations. The presence of DMSO also causes a shrinkage of the multilamellar repeat structures, which appears to be due to reduction in solvent space separating opposing bilayers.

One approach to explaining the influence of DMSO on the thermotropic phase behavior of the phospholipid is in terms of Hofmeister effects. A comparison of the effects of DMSO with a range of other solutes indicates that DMSO behaves as a kosmotrope, or water-structure maker (see review by Collins and Washabaugh, 1985). In the context of lipid bilayer structures, kosmotropes tend to reduce the area of the unfavorable interfaces between aqueous and lipid domains (Koynova et al., 1989; Sanderson et al., 1991). Because the surface area per molecule in the ripple phase is larger than that of the $L_{\beta'}$ phase (Janiak et al., 1979) and is expected to be smaller than that of the L_{α} phase, an increase in temperature of the pre- and main transitions upon addition of DMSO to the solvent is entirely consistent with its action as a kosmotrope.

The present data indicating that DMSO is in close proximity to the polar group of the phospholipid suggest that direct interactions between DMSO and the lipid cannot be excluded. An increase in the motional freedom of head groups during the pre-transition and main transition has been observed (Marsh, 1980), but this effect makes only a minor contribution to the transition enthalpy (McDaniel et al., 1983). If a direct interaction between DMSO and the head groups of the phospholipid constrains the motional freedom of the head group, i.e., enhances interactions be-

tween the head groups, this could also result in an increase in the phase transition temperatures.

The calculated electron density profiles shown that DMSO causes a reduction in dimension of the fluid layer separating the bilayers of multilamellar liposomes. One of the unique characteristics of multilayer structures of phosphatidylcholines dispersed in water is the finite distance of the fluid layer separating opposing bilayers. Many experimental and theoretical contributions have been reported to address the various kinds of forces between bilayers (see, for example, Lis et al., 1982; Cevc, 1991b). The main forces acting between membrane surfaces are attractive van der Waals forces and repulsive electrostatic, undulation, and hydration forces (Marrink et al., 1993; Seddon and Templer, 1993). Electrostatic and undulation forces predominate when the surface separation is greater than the diameter of about 10 solvent molecules (Marrink et al., 1993), whereas the hydration force assumes greater significance as the bilayers come into closer proximity. The repulsive electrostatic force for the non-net-charged DPPC is taken to be negligible (Vierl et al., 1994). Furthermore, because the bilayers are separated by a relatively short distance, van der Waals forces and the hydration force will dominate the interactions between bilayers.

A simple (nonretarded) form of van der Waals force per unit area is expressed in Eq. 2 (Seddon and Templer, 1993):

$$P_a = -H/(6\pi)[d_f^{-3} - 2d^{-3} + (d + d_l)^{-3}] \qquad (2)$$

where H is the Hamaker constant and $d_f = d - d_l$ is the thickness of the solvent layer. By contrast, the origin and theoretical expression of the repulsive hydration force are more problematic (Marrink et al., 1993; McIntosh and Simon, 1994). Nevertheless, it is generally the form of Eq. 3 when determined experimentally (Lis et al., 1982; McIntosh et al., 1989):

$$P_h = P_0\exp(-d_f/\lambda) \qquad (3)$$

where P_0 is a constant and λ the exponential decay constant. For DPPC dispersed in excess water, Lis et al. (1982) found numerical values for a decay constant of 2.1 nm and $P_0 \approx 7 * 10^8$ pa, $H = 6.1 * 10^{-21}$ J. With these constants and a knowledge of the thickness of the lipid bilayer $d_l = 4.13$ nm, obtained from the electron density calculations, the two functions can be related to the fluid phase separation, d_f, within the range of 0.3 to 4.0 nm. These data are presented in Fig. 7, *A* and *B*, in the form of natural logarithms. The point of intersection between the two functions represents the equilibrium lipid surface separation. The value of 2.1 nm for d_f is very close to the experimental result of 2.22 nm, attesting to the validity of the model and the constants employed in the calculations.

It may be expected that a modulation of the properties of solvent layer will result in changes of these constants and thus lead to a shift in the equilibrium of repulsive and attractive forces causing in a change in d_f. McIntosh et al.

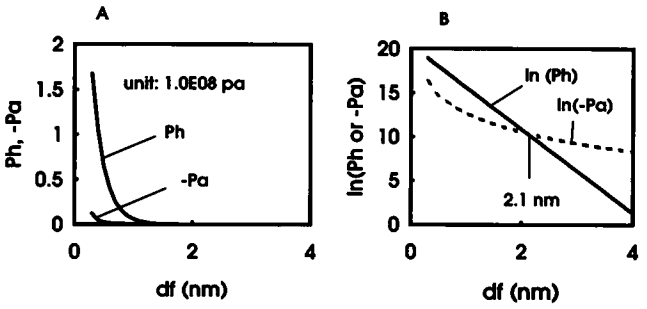

FIGURE 7 (*A*) Attractive force (van der Waals force, P_a) and hydration force (P_h) per unit area vs. fluid layer separation of the DPPC aqueous dispersion.Units of the forces per unit area are in $10^8 N \cdot m^{-2}$ (or pascal). (*B*) Plot of the natural logarithm of the data in (*A*).

(1989) have considered such modulations resulting from replacement of water with formamide or 1,3-propanediol. A continuous expansion of the fluid phase was observed. By contrast, the present study clearly indicates a shrinkage of the fluid phase in the presence of DMSO.

According to Israelachvili (1985), the Hamaker constant for the DPPC-aqueous DMSO system can be expressed as

$$H \approx \frac{3kT}{4}\left(\frac{\varepsilon_l - \varepsilon_f}{\varepsilon_l + \varepsilon_f}\right)^2 + \frac{3h\nu_e}{16\sqrt{2}}\frac{(n_l^2 - n_f^2)^2}{(n_l^2 + n_f^2)^{3/2}} \qquad (4)$$

where ϵ_l, ϵ_f, n_l, and n_f are the static dielectric constants and the refractive indexes of the lipid and fluid layers, respectively, $kT = 4.114 * 10^{-21}$ J at 25°C, and $h\nu_e$ (about $2 * 10^{-18}$ J) is the ionization energy of the bilayer (McIntosh et al., 1989). The first term in Eq. 4 is the zero-frequency dielectric contribution. Assigning a value for the dielectric constant of lipid, ϵ_l, of 2 (McIntosh et al., 1989), a decrease in ϵ_f from 80.2 to 78.5 (Martin and Hauthal, 1971) by the replacement of pure water with DMSO solution (mole fraction of 0.11) results in a decrease in the contribution of static dielectric constant of only about 0.2%. By contrast, the predominant influence on the Hamaker constant arises from the second term, the higher frequency contribution. The refractive indexes of lipid bilayer, water, and DMSO are 1.45 (McIntosh et al., 1989), 1.333, and 1.477, respectively, and the refractive index of the DMSO solution ($x = 0.11$) was taken as 1.3488 with an ideal-mixing approximation. The contribution of the second term of Eq. 4 will, therefore, decrease by about 25% if water is replaced by the DMSO solution. This means that the attractive force per unit area will decrease significantly. This is illustrated in Fig. 8.

The evaluation of the constant P_0 is more problematic. Several factors, including the polarization of water by surface charges or dipoles on the polar head groups and the rearrangement of the hydrogen-bonding pattern of water (McIntosh and Simon, 1994), influence the evaluation of P_0. Cevc and Marsh (1985) considered P_0 to be a function of dielectric constant of the solvent, the permittivity of free space, and the polarizing electric field. Nevertheless, the

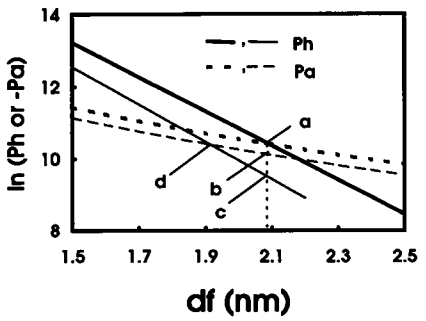

FIGURE 8 Logarithm plot of the hydration force (solid lines) and the attractive force (broken lines) of DPPC dispersions in water (thick lines) and DMSO solution ($x = 0.11$) (thin lines). Units of the forces per unit area are in $N \cdot m^{-2}$. See text for details.

hydration force equation in the presence of DMSO must pass through the point *d* in Fig. 8 because of a reduction in the dimension of the fluid phase. To aid a general discussion of the origin of the shrinkage in the dimension of the fluid phase, a curve of hydration force per unit area is drawn schematically in Fig. 8 with the same P_0 value as pure water but an adjusted decay constant (0.19 nm). Thus, the introduction of DMSO ($x = 0.11$) results in a small decrease in the attractive force (point *a* to point *b*) and a larger decrease in the repulsive force (*a* to *c*). It is the imbalance of these two forces that leads to the shrinkage in dimension of the fluid phase to a point where a new equilibrium distance separating the bilayers is reached (*d* in Fig. 8).

Although the curves in Fig. 8 are only qualitative, the trends revealed by the analysis allow some generalizations to be made about the interaction forces that are operating in the system. By combining all of the factors affecting the attractive or repulsive forces except the fluid phase dimension, i.e., the properties of solvent, lipid assembly, and interface between them, in one imaginary parameter (σ), the two forces per unit area may be expressed as $P_a = F_a(\sigma, d_f)$ and $P_r = F_r(\sigma, d_f)$. Thus two families of curves corresponding to the two forces can be drawn in a manner similar to that illustrated in Fig. 8. The intersection between respective curves at any particular σ will govern the characteristics of the dispersion.

Considerable effort has been devoted to explaining the origin of the ripple phase in diacylphosphatidylcholines (Honda and Kimura, 1991; Cevc, 1991; Marek, 1992). By calculation of the intermolecular van der Waals and Coulomb energy, Marek (1992) demonstrated the importance of steric repulsion between phosphate and choline groups of DPPC molecules in the formation of the ripple phase. Cevc (1991) emphasized the interfacial tendency for a lateral expansion (the head-water-head repulsion) to be the driving force and pointed out the role of hydration in the development of the ripple structure. He introduced a parameter to describe the interfacial hydrophilicity in his model, and the effect of DMSO on the pre-transition reported in the present study is likely to result from a change in this parameter because it is likely to induce changes in local excess charges or changes on the dipolar properties of the interface.

Following the pioneering work of Cevc (1991) and Marek (1992), we conclude that three conditions are required for the formation of a ripple phase: (1) intrinsic steric repulsion between the lipid head groups; (2) mildly weak packing on the acyl chains of lipid molecules; and (3) relatively weak interactions between the head groups.

First, the intrinsic steric repulsion that arises from the relatively large size and weak binding of the head group becomes significant at temperatures greater than the sub-transition temperature. Below this temperature, lipid molecules are in a well-ordered form and the steric repulsion is obscured. Second, the lowering of the bilayer hydration free energy due to the surface undulation must compensate for the energy required to allow the hydrocarbon chains to adjust to a new configuration associated with the undulation. If the acyl chains are too long (e.g., >22 carbons) the ripple phase cannot form. In cases where the chains are too short (e.g., <10 carbons), chain melting will coincide with an increase in steric repulsion, which occurs during the sub-transition. This also explains the gradual narrowing of the temperature range between main and pre-transition temperatures. Third, the relatively weak interactions between head groups is actually a consequence of the sufficient hydration of the head groups.

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REFERENCES

Anchordoguy, T. J., C. A. Cecchini, J. H. Crowe, and L. M. Crowe. 1991. Insights into the cryoprotective mechanism of DMSO for phospholipid bilayers. *Cryobiology.* 28:467–473.

Brady, G. W., and D. B. Fein. 1977. An analysis of the x-ray interchain peak profile in dipalmitoylglycerophosphocholine. *Biochim. Biophys. Acta.* 464:249–259.

Bras, W., G. E. Derbyshire, A. J. Ryan, G. R. Mant, A. Felton, R. A. Lewis, C. J. Hall, and G. N. Greaves. 1993. Simultaneous time resolved SAXS and WAXS experiments using synchrotron radiation. *Nucl. Instruments Methods Phys. Res.* A326:587–591.

Cevc, G., and D. Marsh. 1985. Hydration of noncharged lipid bilayer membranes. Theory and experiments with phosphatidylethanolamine. *Biophys. J.* 47:21–32.

Cevc, G. 1991a. Polymorphism of the bilayer membranes in the ordered phase and the molecular origin of the lipid pretransition and rippled lemallae. *Biochim. Biophys. Acta.* 1062:59–69.

Cevc, G. 1991b. Hydration force and the interfacial structure of the polar surface. *J. Chem. Soc. Faraday Trans.* 87:2733–2739.

Chapman, D. 1973. Some recent studies of lipids, lipid-cholesterol and membrane systems. *In* Biological Membranes, Vol. 2. D. Chapman and D. F. H. Wallach, editors. Academic Press, London. 91–144.

Collins, K. D., and M. W. Washabaugh. 1985. The Hofmeister effect and the behaviour of water at interfaces. *Q. Rev. Biophys.* 18:323–422.

Cunningham, B. A., W. Bras, L. J. Lis, and P. J. Quinn. 1994. Synchrotron x-ray studies of lipids and membranes: a critique. *J. Biochem. Biophys. Methods.* 29:87–111.

Franks, N. P., and Y. K. Levine. 1981. Low-angle x-ray diffraction. *In* Membrane Spectroscopy. E. Grell, editor. Springer, Berlin. 437–487.

Füldner, H. H. 1981. Characterization of a third phase transition in multilamellar dipalmitoyllecithin liposomes. *Biochemistry.* 20:5707–5710.

Honda, K., and H. Kimura. 1991. Theory on formation of the ripple phase in bilayer-membranes. *J. Phys. Soc. Jpn.* 60:1212–1215.

Israelachvili, J. N. 1985. Intermolecular and Surface Forces. Academic Press, London.

Janiak, M. J., D. M. Small, and G. G. Shipley. 1979. Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin. *J. Biol. Chem.* 254:6068–6078.

Koynova, R. D., B. G. Tenchov, and P. J. Quinn. 1989. Sugars favour formation of hexoganol (H_{II}) phase at the expense of lamellar liquid-crystalline phase in hydrated phosphatidylethanolamines. *Biochim. Biophys. Acta.* 980:377–380.

Koynova, R. D., B. G. Tenchov, S. Todinova, and P. J. Quinn. 1995. Rapid reversible formation of a metastable subgel phase in saturated diacylphosphatidylcholines. *Biophys. J.* 68:2370–2375.

Lewis, R. N. A. H., N. Mak, and R. N. McElhaney. 1987. A differential scanning calorimetric study of the thermotropic phase behaviour of model membranes composed of phosphatidylcholines containing linear saturated fatty acyl chains. *Biochemistry.* 26:6118–6126.

Lis, L. J., M. McAlister, N. Fuller, R. P. Rand, and V. A. Parsegian. 1982. Interactions between neutral phospholipid bilayer membranes. *Biophys. J.* 37:657–666.

Marek, J. 1992. A difference in the shapes of intermolecular potentials between phospholipid model molecules, relation to the ripple phase. *J. Theor. Biol.* 159:417–429.

Marrink, S. J., M. Berkowitz, and H. J. Berendsen. 1993. Molecular dynamics simulation of a membrane/water interface: the ordering of water and its relation to the hydration force. *Langmuir.* 9:3122–3131.

Marsh, D. 1980. Molecular motion in phospholipid bilayers in the gel phase: long axis rotation. *Biochemistry.* 19:1632–1637.

Martin, D., and H. G. Hauthal. 1971. Dimethyl Sulphoxide. E. Halberstadt, translator. Van Nostrand Reinhold, Wokingham.

Matuoka, S., S. Kato, M. Akiyama, Y. Amemiya, and I. Hatta. 1990. Temperature dependence of the ripple structure in dimyristoylphosphatidylcholine studied by synchrotron x-ray small-angle diffraction. *Biochim. Biophys. Acta.* 1028:103–109.

McDaniel, R. V., T. J. McIntosh, and S. A. Simon. 1983. Nonelectrolytes substitution for water in phosphatidylcholine bilayers. *Biochim. Biophys. Acta.* 731:97–108.

McIntosh, T. J. 1980. Differences in hydrocarbon chain tilt between hydrated phosphatidylethanolamine and phosphatidylcholine bilayers. a molecular packing model. *Biophys. J.* 29:237–246.

McIntosh, T. J., A. D. Magid, and S. A. Simon. 1989. Range of the solvation pressure between lipid membranes: dependence on the packing density of solvent molecules. *Biochemistry.* 28:7904–7912.

McIntosh, T. J., and S. A. Simon. 1994. Hydration and steric pressures between phospholipid bilayers. *Annu. Rev. Biophys. Biomol. Struct.* 23:27–51.

Meyer, H. W., B. Dobner, P. Nuhn, G. Brezesinski, and G. Forster. 1994. Freeze-fracturing for demonstration of ripple modulations in DPPC-bilayer containing cholesterol or different branched-chain phosphatidylcholines. Int. Conf. Electron Microsc. *Paris.* 13:21–22.

Quinn, P. J., H. Takahashi, and I. Hatta. 1995. Characterization of complexes formed in fully hydrated dispersions of dipalmitoylcholine and diacylglycerol. *Biophys. J.* 68:1374–1382.

Sanderson, P. W., L. J. Lis, P. J. Quinn, and W. P. Williams. 1991. The Hofmeister effect in relation to membrane lipid phase stability. *Biochim. Biophys. Acta.* 1067:43–50.

Seddon, J. M., and R. H. Templer. 1993. Cubic phases of self-assembled amphiphilic aggregates. *Phil. Trans. R. Soc. Lond. A.* 344:377–401.

Schneider, M. B., W. K. Chan, and W. W. Webb. 1983. Fast diffusion along defects and corrugations in phospholipid $P_{\beta'}$ liquid crystals. *Biophys. J.* 43:157–165.

Tamura-Lis, W., L. J. Lis, S. Qadri, and P. J. Quinn. 1990. Ethanol-phosphatidylcholine interactions: a real-time x-ray diffraction study. *Mol. Cryst. Liq. Cryst.* 178:79–88.

Tardiu, A., V. Luzzati, and F. C. Reman. 1973. Structure and polymorphism of hydrated chains of lipids: a study of lecithin-water phases. *J. Mol. Biol.* 75:711–733.

Tenchov, B. G., H. Yao, and I. Hatta. 1989. Time-resolved x-ray diffraction and calorimetric studies at low scan rates. 1. Fully hydrated dipalmitoylphosphatidylcholine (DPPC) and DPPC/water/ethanol phases. *Biophys. J.* 56:757–768.

Tenchov, B. 1991. On the reversibility of the phase transitions in lipid-water systems. *Chem. Phys. Lipids.* 57:165–177.

Torbet J., and M. H. F. Willkins. 1976. x-ray diffraction studies of lecithin bilayers. *J. Theor. Biol.* 62:447–458.

Tristram-Nagle, S., R. Zhang, R. M. Suter, C. R. Worthington, W.-J. Sun, and J. F. Nagle. 1993. Measurement of chain tilt angle in fully hydrated bilayers of gel phase of dipalmitoylphosphatidylcholine. *Biophys. J.* 64:1097–1109.

Tsuchida, K., and I. Hatta. 1988. ESR studies on the ripple phase in multilamellar phospholipid bilayers. *Biochim. Biophys. Acta.* 945: 73–80.

Tsvetkova, N., R. Koynova, L. Tsonev, P. Quinn, and B. Tenchov. 1991. Influence of proline on the stability of fully hydrated and freeze-dried lipid phases. *Chem. Phys. Lipids.* 60:51–59.

Veiro, J. A., P. Nambi, L. L. Herold, and E. S. Rowe. 1987. Effect of n-alcohols and glycerol on the pretransition of dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta.* 900:230–238.

Vierl, U., L. Löbbecke, N. Nagel, and G. Cevc. 1994. Solute effects on the colloidal and phase behavior of lipid billayer membranes: ethanol-dipalmitoylphosphatidylcholine mixtures. *Biophys. J.* 67:1067–1079.

Warren, R. C. 1987. Physics and the Architecture of Cell Membranes. Adam Hilger, Bristol.

Wittebort, R. J., C. F. Schmidt, and R. G. Griffin. 1981. Solid-state carbon-13 nuclear magnetic resonance of the lecithin gel to liquid-crystalline phase transition. *Biochemistry.* 20:4223–4228.

Wolfe, D. H., L. J. Lis, O. Kucuk, M. P. Westerman, B. A. Cunningham, S. B. Qadri, W. Bras, and P. J. Quinn. 1992. Phase transitions between ripple structures in hydrated phosphatidylcholine-cholesterol multilamellar assemblies. *Phys. Rev. Lett.* 68:1085–1088.

Yu, Z. W., and P. J. Quinn. 1994. Dimethyl sulphoxide: a review of its applications in cell biology. *Biosci. Rep.* 14:259–281.