Lipid Membrane Structure and Interactions in Dimethyl Sulfoxide/Water Mixtures

V. I. Gordeliy,* M. A. Kiselev,# P. Lesieur,§ A. V. Pole,# and J. Teixeira¶

*IBI-2, Structural Biology, Forschungszentrum Jülich, D-52425 Jülich, Germany; #Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Moscow District, 141980 Russia; §Laboratory for Use of Electromagnetic Radiation, Orsay, France; and ¶Leon Brillouin Laboratory, CE de Saclay, France

ABSTRACT In this paper we have investigated via x-ray diffraction the influence of dimethyl sulfoxide (DMSO), known for its biological and therapeutic properties, on the structure of lipid membranes of dipalmitoylphosphatidylcholine (DPPC) in excess of the solvent (DMSO/water) at mole DMSO fractions $X_{DMSO} \in (0.1)$ and under equilibrium conditions. At small $X_{DMSO} \leq 0.133$ the repeat distance d is reduced remarkably, whereas wide-angle x-ray diffraction pattern remains almost unchanged with the increase in X_{DMSO}. It agrees well with previous study (Yu and Quinn, 1995). At $0.133 < X_{DMSO} < 0.3$ the repeat period d reduces slowly; however, an orthorombic in-plane lattice of hydrocarbon chains transfers to a disordered quasihexagonal lattice. The increase in X_{DMSO} from 0.3 up to ~0.9 leaves d almost unchanged, whereas it leads to less disordered packing of hydrocarbon chains. At $X_{DMSO} \approx 0.9$, $L_{\beta'}$ phase transfers into interdigitated phase. The chain-melting phase transition temperature of DPPC membranes increases by several degrees with the increase of DMSO concentration. It points to a strong concentration-dependent solvation of membrane surface by DMSO. Thus DMSO strongly interacts with the membrane surface, probably displacing water and modifying the structure of the lipid bilayer. It appears to determine some of the properties of DMSO as a biologically and therapeutically active substance.

INTRODUCTION

Water is generally believed to determine many of the properties of biological objects, including biological membranes. The lipid bilayer, being the main structural component of membranes, is responsible for many of its properties (Gennis, 1989). The structural organization of the lipid-water system has so far been rather well studied (Gennis, 1989; Schipley, 1973; Tardieu et al., 1973). Paradoxically, with such a large number of papers in this field of research, many of the fundamental properties of lipid membranes have as yet defied explanation (Zaccai et al., 1994).

A primary consideration, being at this time in the focus of investigation, is the mechanism of short-range intermembrane repulsive forces (often called hydration forces), dominating through distances up to 30 Å (Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992). Major items to be discussed here refer to how the polar membrane surface polarizes water, and whether the magnitude of water polarization is sufficient to produce huge repulsive forces acting at short intermembrane distances (Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992). Or, perhaps, "hydration" forces are of an entropic nature because of out-of-plane thermal fluctuations of lipid molecules (Israelachvili and Wennerström, 1992).

Received for publication 25 September 1995 and in final form 6 May 1998.

Address reprint requests to Dr. V. I. Gordeliy, IBI-2, Structural Biology, Forschungszentrum Jülich, D-52425 Jülich, Germany. Tel.: 49-2461-612034; Fax: 49-2461-612020; E-mail: g.valentin@fz-juelich.de.

Dr. Gordeliy's permanent address is Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Moscow District, 141980 Russia.

0006-3495/98/11/2343/09 $2.00

Considerable study is being given to the impact upon membrane structure of nonwater polar solvents. The investigation of peculiarities and limits of membrane existence in various nonwater solvents is interesting in itself. But most intriguing here is to use these solvents as a way to find a better explanation of the role of water in membrane stabilization and interaction (Vierl et al., 1994).

An effort to understand the mechanism of "hydration" forces by comparison studies of intermembrane interactions in water, formamide, and 1,3-propandiol was made in the work of McIntosh et al. (1989). The decay length λ of the forces was related to the packing density of solvent molecules in intermembrane space. A nearly proportional relationship was made apparent between the exponential factor P_0 ($P_{hyd} \approx P_0 \exp[-d_w/\lambda]$, where d_w is the intermembrane distance) and the square of the dipole potential at the membrane surface (more exactly, the solvation potential; Cevc and Marsh, 1985; McIntosh et al., 1989). In the paper of McIntosh et al. (1989), no correlation was observed between the "hydration" forces and the dielectric permeability ϵ of the solvent. All of this was treated then as an argument in favor of the polarization mechanism of "hydration" forces. But now a definite correlation between the magnitude of λ and that of surface energy of the solvent is discussed (Israelachvili and Wennerström, 1992). To understand the nature of short-range intermembrane repulsive forces further, investigation is imperative (Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992; McIntosh and Simon, 1994; McIntosh et al., 1995; Simon et al., 1995; Gordeliy, 1996; Gordeliy et al., 1996a,b).

Recently lipid membrane structure has been investigated at small mole fractions (up to 0.133) of DMSO in water (Yu and Quinn, 1995). It was shown in particular that DMSO

influences intermembrane interactions in a way that is different from that of some other solvents.

In this paper the purpose is to investigate the impact of dimethyl sulfoxide (DMSO) on the structure and interaction of lipid membranes over a wide range of molar ratios of DMSO/water and temperatures as well.

DMSO is attractive as a nonwater solvent when intermembrane interactions are to be probed: DMSO makes hydrogen bonds with water molecules (it is an acceptor of hydrogen bonds), and the DMSO concentration dependence of the physical characteristics of a DMSO/water mixture is of a nonmonotonic nature (Doucet et al., 1965; Tommila and Pajunen, 1969).

It is worthy of notice that DMSO mole ratios higher than 0.133, i.e., beyond the range that was studied (Yu and Quinn, 1995), are of great interest as well. It is well known that many physicochemical parameters of the DMSO/water mixture itself exhibit their maximum or minimum at $X_{DMSO} \approx 0.3 \div 0.4$ (Gugenheim, 1952).

What also counts is that the structure of dimethyl sulfoxide-water mixtures was studied at the molecular level by means of neutron diffraction (Soper and Luzar, 1992). In this paper water structure was not found to be strongly affected by the presence of DMSO. However, the percentage of water molecules that are hydrogen bonded to themselves was substantially reduced compared to pure water. DMSO is able to make hydrogen bonds (Soper and Luzar, 1992). It is of interest to find out how water and DMSO compete with each other for a direct interaction with membrane surface. The interaction of DMSO and biological membranes may have an important influence on membrane function.

There is also a biological and medical interest in the study of DMSO/water/lipid systems, particularly at higher X_{DMSO}. Indeed, in the paper of Pande et al. (1989), dimethyl sulfoxide-induced changes were detected in bacteriorhodopsin of purple membranes. The changes began at 40% DMSO and resulted in complete conversion of dark-adapted bR (bR_{560}) to a species absorbing maximally at $\lambda = 480$ nm (bR_{480}) at 60% DMSO.

DMSO also has widespread pharmacological applications, and again it is used at high concentrations as well (Hagemann et al., 1970). It is used as drug carriers to cells (Wood and Wood, 1975). Dimethyl sulfoxide is one of the best cryoprotectors (Lowelock and Bishop, 1979).

The dimethyl sulfoxide molecule (C_2H_6SO) has six hydrogens that offer possibilities of looking at the interaction of DMSO with the membrane surface by means of neutron scattering with isotopic substitution of deuterium for hydrogen.

The present paper offers first results in the study of membrane structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) in an x-ray diffraction experiment at the following mole fractions X_{DMSO} of DMSO/water: 0:0.2; 0.5:0.8, and 1, at the temperature interval from 20°C to 85°C. All measurements were performed in excess solvent.

At $T = 20$°C the study was carried over a wider range of concentrations ($X_{DMSO} \in (0.1)$).

MATERIALS AND METHODS

Materials

DPPC (over 99% pure) was obtained from SERVA (Germany). DMSO (over 99% pure) was produced by MERK (Darmstadt, Germany). Water (18 MΩ/cm) was obtained with the help of Millipor.

Specimen preparation

The given amount of DMSO in a DMSO/water solution was mixed with the lipid in 1:1 w/w solvent/lipid ratio and placed in an x-ray quartz capillary with 1.5 mm diameter and 0.01 mm wall thickness (W. Müller, Berlin, Germany). The capillary was hermetically sealed, and the specimens were held at the temperature higher than that of phase transition for several hours.

Small-angle x-ray measurements were carried out at the small-angle instrument D24 of the synchrotron source DCI (Laboratory for Use of Electromagnetic Radiation, Orsay, France). A one-dimensional position-sensitive detector was used in this case.

Wide-angle measurements were performed at an x-ray diffractometer adapted for membrane studies (Gordeliy et al., 1994). At this diffractometer a part of small-angle measurements were made to check the reproducibility of the results. The x-ray diffractometer has a Cu anode tube as a radiation source. Bragg-Brentano focusing and two slits are placed at a distance of 10 mm from each other, close to the output window of the tube for x-ray beam formation. An additional slit is placed just before the sample to suppress the scattering from the boundaries of the second slit. A single detector is used to detect the scattered x-rays. A θ-2θ scan is carried out in the horizontal plane (with the rotation of the sample and the detector around the vertical axis).

In both cases measurements were made at a Ni-filtered CuKα radiation x-ray source with 1.54-Å wavelength.

The specimen container was maintained at a constant temperature by using liquid thermostat to the precision of ±0.5°C and controlled by a thermocouple.

The repeat periods d of the multilayer structure were determined from the positions of diffraction peaks (by using the Bragg equation: $2d \times \sin\theta = \lambda$, where θ is half the scattering angle).

From integral intensities the modules of structure factors were calculated by the formula $|F(h)| \approx \mathrm{I}(h) \times L^{-1/2} \times P^{-1/2}$, where h is the order of diffraction reflection, L is the Lorentz factor, proportional for unoriented specimens (as was the case) at small angles to h^2, and P is the polarization factor equal to $0.5(1 + \cos^2\theta)$ (Blaurock and Worthington, 1966).

For the centrosymmetric structure, which is the monolipid bilayer, the electron density profile along the normal of membrane plane (the x axis) was calculated by the equation

$$\rho(x) = \sum_{\mathrm{h}} F(h) \cdot \cos\frac{2\pi h x}{d} \qquad (1)$$

The phases of structural factors, being for centrosymmetric structures equal to ±1, were determined by the technique developed by Worthington (1969), King and Worthington (1971), and Worthington et al. (1973).

The measurements have been made with DPPC membranes at different concentrations of the solvents (i.e., different swellings). It allows one to apply Shannon's sampling theorem to the set of structure factor data. The structure factors for different solvents were normalized in accordance with the standard procedure. The details of this approach to determining the phases of structure factors are described elsewhere (see, for instance, Franks, Levine, 1981; McDaniel et al., 1983; McIntosh et al., 1989; Yu and Quinn, 1995). The phases used for the calculation of the electron density profiles were −, −, +, − ($L_{\beta'}$ phase) and −, −, +, + ($L_{\beta I}$-interdigitated phase).

RESULTS

The effect of DMSO concentration: gel phase

A typical diffraction pattern of the DPPC/DMSO/water mixture corresponding to a multilamellar structure is shown in Fig. 1. In the given case it is related to the DMSO concentration $X_{DMSO} = 0.05$ (the mole fraction of DMSO in water/DMSO mixture) and the temperature $T = 30°C$. The two central peaks result from an incomplete blocking of a direct x-ray beam. The diffraction pattern is not symmetrical, because the center of the detector has been shifted relative to the direct x-ray beam.

The dependence of the repeat period d on the DMSO concentration in water at the temperature $T = 20°C$ is presented in Fig. 2. One can see four characteristic behavior regions of the membrane structure parameters. In the first region at ratios $0 \leq X_{DMSO} \leq 0.133$ one observes a quick, almost monotonic decrease of the repeat period from 64 Å (for pure water) to 59 Å at $X_{DMSO} = 0.1$ with the DMSO concentration increasing. The same was observed by Yu and Quinn (1995).

The second region, beginning at $X_{DMSO} \approx 0.133$ and continuing up to $X_{DMSO} = 0.3$, is related to small changes in the repeat distance. The repeat period is monotonously decreasing here by ~1 Å down to 58 Å at the DMSO mole fraction $X_{DMSO} = 0.3$.

At $0.3 \leq X_{DMSO} < 0.9$ the repeat distance is almost constant.

Region four at $0.9 \leq X_{DMSO} < 1$ seems to be related to a sharp change in membrane structure with the decrease in repeat period down to 52 Å.

The influence of DMSO on lipid membrane properties is strikingly different from that of some other solvents. For instance, the repeat distance of membrane is monotonously increasing with the increase in glycerol concentration (at small fractions of glycerol in water/glycerol mixture) (McDaniel et al., 1983; McIntosh et al., 1989), whereas the both solvents are often considered similar in terms of their influence on the cell (Wood and Wood, 1975).

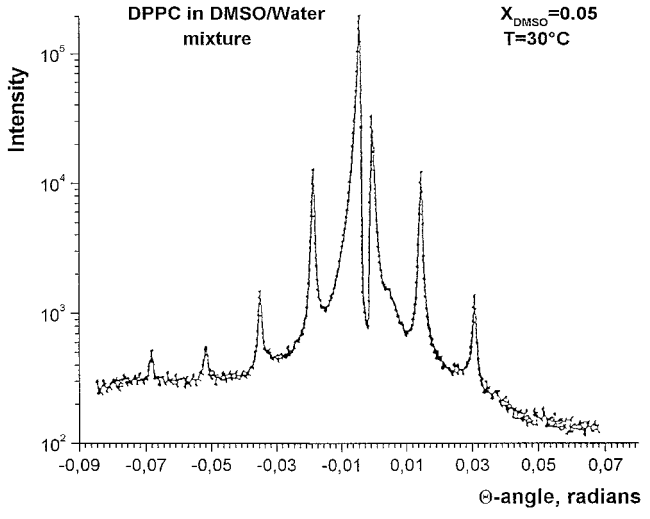


FIGURE 1 X-ray diffraction pattern from DPPC/DMSO/water mixture at DMSO concentration $X_{DMSO} = 0.05$ and $T = 30°C$. Two central peaks result from an incomplete blocking of a direct x-ray beam. The pattern is not symmetrical. The center of a one-dimensional detector was shifted relative to the x-ray beam.

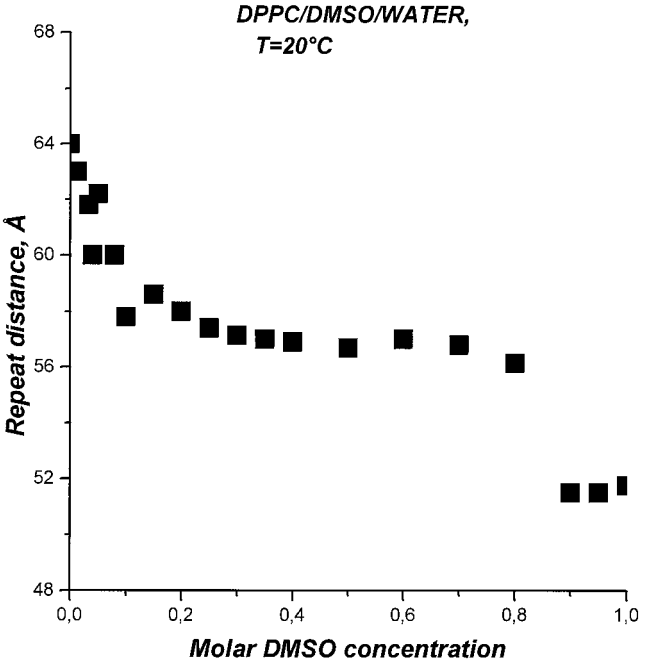


FIGURE 2 The dependence of the repeat distance d of DPPC membranes on the DMSO concentration at $T = 20°C$.

To check the reproducibility of experimental data, we performed x-ray diffraction measurements with other DPPC samples at certain characteristic concentrations of DMSO (data not shown). The values of the repeat distances and phase transition temperatures are the same within experimental accuracy for two sets of samples. Moreover, our data are in excellent agreement with recent measurements of DPPC membranes at small concentrations of DMSO (Yu and Quinn, 1995).

The phase state of the membrane can easily be identified by wide-angle diffraction from the hydrocarbon chains of lipid molecules (Tardieu et al., 1973).

Moreover, a large body of experimental data (Schipley, 1973; Tardieu et al., 1973; McIntosh et al., 1989; Kirchner and Cevc, 1993) shows that phase transitions in multilamellar membranes are always followed by a sharp change in other structural parameters as well. For instance, an abrupt change in the repeat distance (in the gel-phase of the membrane) down to ~52 Å in excess water is usually related to a transition to the interdigitated ($L_{\beta I}$) phase (with the penetration of hydrocarbon chains of one monolayer of the bilayer into the other; Kirchner and Cevc, 1993; Simon et al., 1988; Kim et al., 1987). As will be seen from the following account at the DMSO fractions, $X_{DMSO} = 1$ (in pure DMSO) membranes really form the $L_{\beta I}$ phase.

The effect of DMSO concentration: liquid phase

The repeat distance values have been taken at temperatures just above the critical region of phase transition. As in the gel phase, the repeat distance *d* decreases abruptly with increasing DMSO fraction up to $X_{DMSO} = 0.2$ and then continues to decrease slowly.

The data for the DMSO mole fraction dependence of the repeat period in the liquid phase (L_α phase) are included in Table 1.

As in the gel phase, the repeat distance *d* decreases sharply (by 8 Å) with the increase in mole fraction of DMSO from 0 up to 0.2. With a further increase in DMSO concentration, *d* is decreasing monotonously from 50 Å at $X_{DMSO} = 0.2$ down to 41 Å at $X_{DMSO} = 1$. As the interdigitated phase has never been observed in the liquid phase of DPPC membrane, we assume that a reduction in lipid bilayer thickness and solvent layer thickness both are responsible for such a dramatic change of d.

The temperature dependence of membrane structural parameters

Temperature-dependent changes of the repeat period *d* at the DMSO mole fractions $X_{DMSO} = 0.2$, 0.5, 0.8, and 1.0 have been measured. At all concentrations a similar qualitative change in the structural parameters of membrane is apparent with the temperature variation. A typical dependence (at $X_{DMSO} = 0.5$) is shown in Fig. 3. At first, within the temperature range from $T = 20°C$ to $T \approx 45 \div 60°C$, the repeat distance in the gel phase remains unaffected. The structural parameters of membrane in pure water behave in just in the same way (Inoko and Mitsui, 1978; Gordeliy et al., 1993; Honger et al., 1994). The next region, that of the main phase transition, the gel-liquid crystalline phase (the phase transition temperature T_m ranging from 41°C to 60°C, according to the DMSO concentration) is marked by a drastic decrease in the repeat distance. And the last region, following that of phase transition, corresponds to a smoothly decreasing repeat distance. The thermal compressibility coefficient of the bilayer ranges within 0.12 ± 0.03 Å/K^{-1} for all of the DMSO concentrations, which is about the same as that observed for DPPC in pure water (Inoko and Mitsui, 1978).

The concentration dependence of the phase transition temperature is presented in Fig. 4.

The phase transition temperature is increasing from 41°C at $X_{DMSO} = 0$ to 49°C at $X_{DMSO} = 0.2$ and appears to remain almost unchanged up to the values of X_{DMSO} corresponding to the transition to the $L_{\beta I}$ (interdigitated) phase.

TABLE 1 The dependence of repeat distance *d* of DPPC membrane in liquid phase on mole fraction X_{DMSO} of DMSO in water/DMSO mixture

X_{DMSO}	0	0.2	0.5	0.8	1
d (Å)	58	50	47	44	41

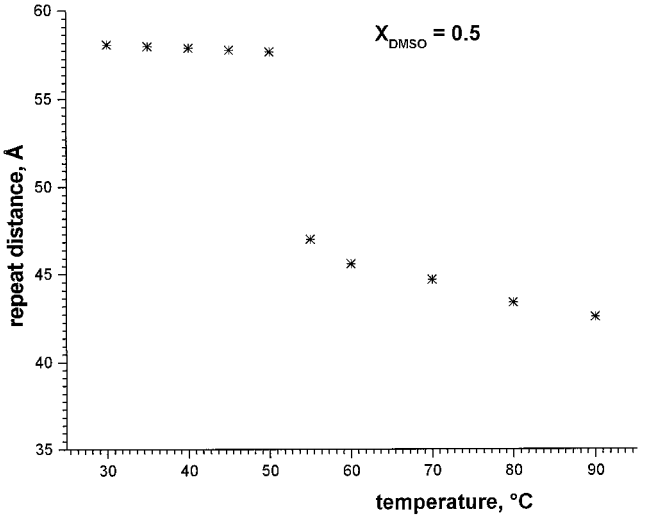


FIGURE 3 The temperature dependence of DPPC membrane repeat distance at the concentration of DMSO $X_{DMSO} = 0.5$.

The phase transition temperature from the $L_{\beta I}$ to the L_α phase is much higher, equal to $77 \pm 1°C$. The explanation of this phenomenon has been given previouosly (Simon and McIntosh, 1984).

The bilayer electron density profiles

To elucidate the nature of an abrupt change in the repeat distance in the gel phase at the DMSO concentration $X_{DMSO} = 1$, electron density profiles $\rho(x)$ (see Materials and Methods) were calculated and are represented in Fig. 5. The zero coordinate corresponds to the center of the hydrophobic part of the membrane. As may be seen, the electron density profiles $\rho(x)$ at all concentrations with the exception of $X_{DMSO} = 1$ are in correspondence with those for membranes in the L_β phase, with a characteristic dip in the center

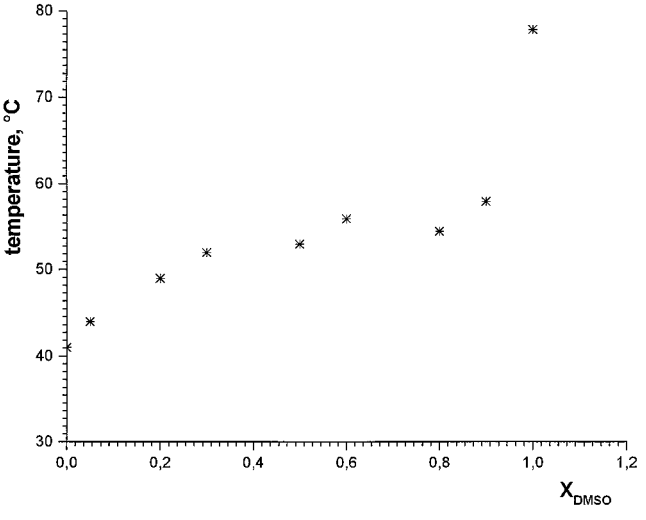


FIGURE 4 The dependence of the chain-melting phase transition temperature on the DMSO concentration.

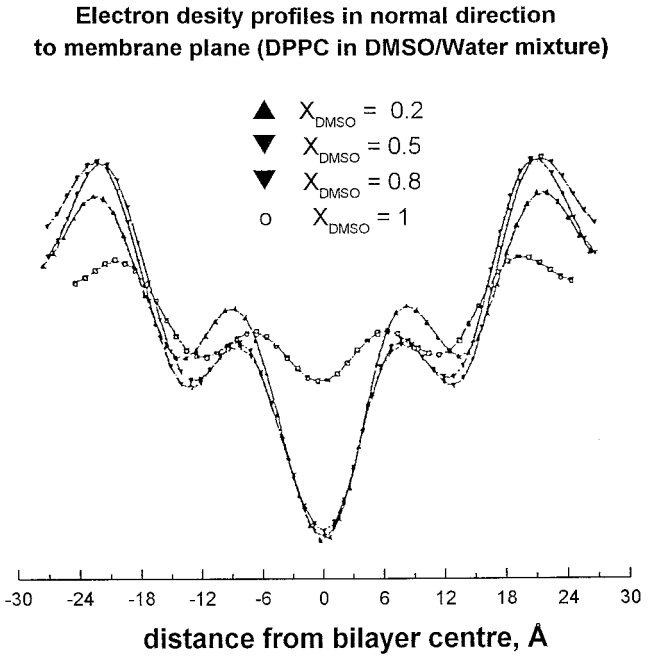


FIGURE 5 Electron density profiles of DPPC membranes at different concentrations of DMSO at the temperature $T = 30°C$. The scales are different for different profiles.

corresponding to a low electron density in the area of end methyl groups of the hydrocarbon chains of lipid molecule (Ranck et al., 1977). This minimum in the center of the bilayer is missing from the electron density profile for the 100% DMSO concentration, and the electron density for the polar part is lower. The statistical accuracy of measured integral intensities of diffraction peaks at all mole fractions of DMSO was better than 1% for the first and second diffraction orders and better than 3% for the highest orders. It means that the dramatic change in the DMSO profile at $X_{DMSO} = 1$ cannot be accounted for by the errors in determination of structure factors. The electron density profile of this kind corresponds to the interdigitated phase (Ranck et al., 1977; McIntosh et al., 1983). Wide-angle diffraction studies indicated a narrow symmetrical diffraction peak related to $Q = (2\pi/4.25)$ Å^{-1} (Fig. 6 *d*), which also proves that the membrane is really in the $L_{\beta I}$ phase and the hydrocarbon chains of lipid molecules are located perpendicular (or nearly perpendicular) to the membrane plane. According to the latest treatment of the nature of the $L_{\beta'} \rightarrow L_{\beta I}$ transition (Pascher et al., 1981), it is caused by a large swelling of the polar part of membrane along its plane. It results in an increase in the tilt angle of hydrocarbon chains to a certain critical maximum possible in the $L_{\beta'}$ phase ($\approx$55°) (Vierl et al., 1994; Pascher et al., 1981). (Here we assume that the mechanism of this transition does not depend on the kind of solvent.) It means that the polar head solvation in the membrane plane in the case of DMSO is higher than in water, which in turn suggests that there is a stronger interaction between DMSO and the membrane surface. However, this interaction seems to be weaker than in the case of ethanol, which at the concentration $C_{ETH} = 1.2$ M transfers membrane into the $L_{\beta I}$ phase, where with a further increase in C_{ETH}, the hydrocarbon chains tilt relative to the normal of the bilayer plane (Vierl et al., 1994).

The DMSO interaction with the membrane surface is confirmed by a change in the phase transition temperature (Fig. 4). But what is unusual is that the temperature of the phase transition $L_{\beta'} \rightarrow L_{\alpha}$, followed by the melting of hydrocarbon chains, is not decreasing but increasing.

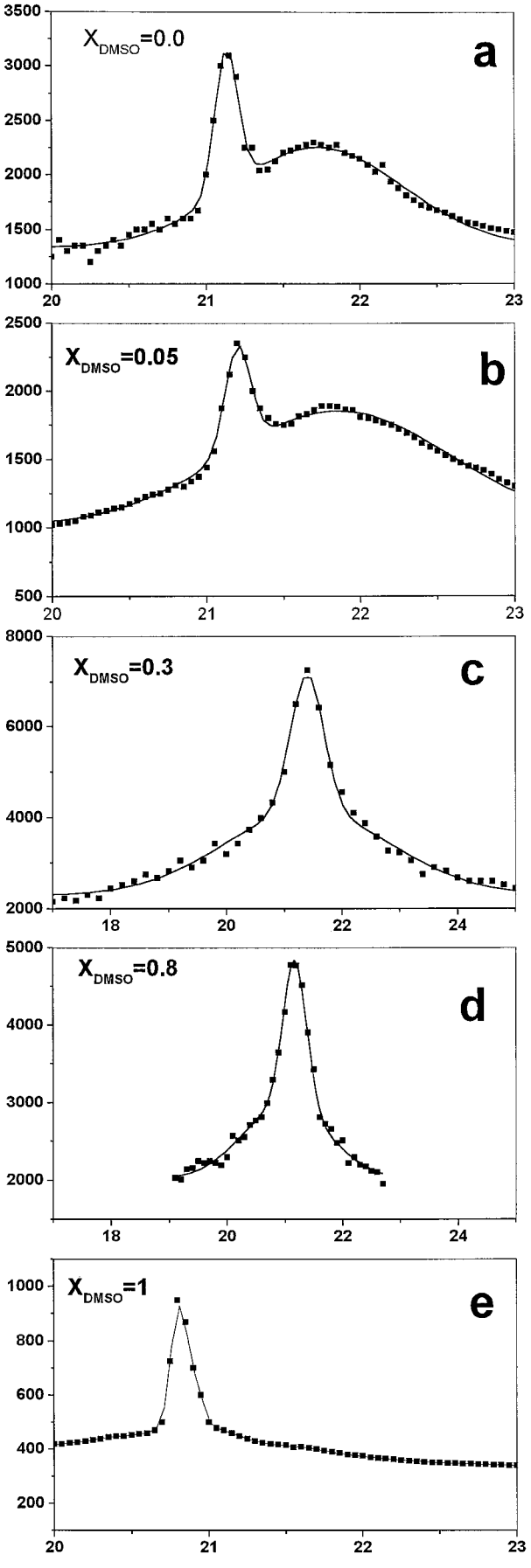
Intermembrane distance in the presence of DMSO

And yet the most intriguing phenomenon is a rapid decrease in the repeat distance with increasing DMSO concentration. It changes from $d = 64$ Å in pure water down to $d = 58$ Å already at $X_{DMSO} = 0.1$. On the addition of such solvents as glycerol, sucrose, and ethanol, d is, to the contrary, increasing (at least at small concentrations of these solvents) (Vierl et al., 1994; McIntosh et al., 1989).

To understand the influence of DMSO on intermembrane interactions, it is necessary to know the intermembrane distance d_w at various concentrations of the solvent. One can use the electron density profile to determine the intermembrane distance (McIntosh et al., 1989). This model calculation of intermembrane distance in the multilayer structure of DPPC in excess water leads to $d_w = 12$ Å, where $d_w = d - d_l$, and the bilayer thickness d_l is calculated in accordance with McIntosh et al. (1989) as $d_l = d_{pp} + 10$ (Å) = 52 Å (McIntosh et al., 1989), and $d_{pp} = 42$ Å (d_{pp} is the distance between the peaks of electron density profiles). If we assume that the maxima of electron density profiles correspond to the same molecular group in the polar part of the membrane as in the case of membranes in pure water and the difference in the conformation of the polar part of the membrane is negligible, then from our data (Fig. 5) it follows that at $X_{DMSO} = 0.1$, $d = 58$ Å, and $d_{pp} = 42$ Å, and consequently the bilayer thickness $d_l = 52$ Å. The equilibrium intermembrane distance is equal to $d_w = d - d_l = 6$ Å.

Electron densities of DMSO and water are almost the same (0.356 and 0.333 e/Å^3, respectively). This means that replacement of water by DMSO will not change the electron density profile shape considerably (if the assumptions made above are valid). However, if there is a preferred orientation of DMSO molecules near the membrane surface, then, because of the S atom, it can shift the maxima in density profiles toward the solvent region. The value of the shift cannot be larger than $\sim$1.5 Å (the approximate distance between S and O atoms). Thus, even when we take into account this hypothetical shift, we come to the same conclusion: intermembrane distance is reduced in the presence of DMSO.

Another approach was used to get information about d_w at small concentrations of DMSO (Yu and Quinn, 1995). The authors of this work came to the same conclusion (their approach will be considered in the following section).



Wide-angle diffraction and interaction between DMSO and membrane surface

The interaction between DMSO and membrane is not trivial. As indicated earlier, the temperature of phase transition for membranes in the presence of DMSO is increasing, and the area per polar head of lipid molecule is expanding. Normally with the area per polar head increasing, the phase transition temperature decreases (Cevc and Marsh, 1987). This "discrepancy" cannot be accounted for by our using a indirect evidence of the area per lipid molecule expanding in a bilayer plane. It will be recalled that we came to such a conclusion supposing that it is an indispensable condition of the phase transition $L_{\beta'} \rightarrow L_{\beta I}$. However, wide-angle x-ray diffraction from hydrocarbon chains of lipid molecules unequivocally shows the tilt of hydrocarbon chains, which increases somewhat with increasing DMSO concentration (Fig. 6, *a–c*). Fig. 6 *a* shows wide-angle diffraction from DPPC membranes in pure water. The sharp reflex corresponding to the lattice constant of 4.19 Å and an additional wide peak (d = 4.09 Å) ("the shoulder" on the side of large scattering angles) are interpreted according to the method of Tardieu et al. (1973) and Janiak et al. (1979) as diffraction on quasihexagonal ("disturbed") lattice from the hydrocarbon chains, tilted in relation to the normal of the bilayer plane. In this case the additional "shoulder" results from the disruption of hexagonal lattice, and the width of the (11) diffraction peak is dependent on the tilt angle of hydrocarbon chains (Tardieu et al., 1973; Janiak et al., 1979). Fig. 6 *b* presents the diffraction picture at $X_{DMSO} = 0.05$. It is evident that DMSO does not affect the lateral structure of membranes at such low concentrations. The sharp diffraction peak (d = 4.18 Å) becomes a little smaller, whereas the width of the "shoulder" (d = 4.08 Å) increases just a little as compared with Fig. 6 *a*. It was also shown that at $X_{DMSO} = 0.133$ the wide-angle diffraction pattern remains almost the same as that for DPPC membranes in pure water (Yu and Quinn, 1995). This fact was used to conclude that the tilt of the chain of lipid molecules in the gel phase of DPPC membranes remains almost the same in the presence of DMSO, and the thickness of the bilayer consequently remains constant. This experimental fact does allow one to state that the reduction of intermembrane distance accounts for the decrease in the repeat distance with the increase in DMSO concentration. However, at higher concentrations of DMSO ($X_{DMSO} \geq 0.133$) this approach to the determination (estimation) of intermembrane distance no longer works. Considerable changes in

FIGURE 6 Wide-angle diffraction from hydrocarbon chains of DPPC membranes in DMSO/water mixtures at different concentrations of DMSO. The vertical axis corresponds to the scattering intensity, the horizontal axis to the scattering angle 2θ. The diffraction peaks are fitted by one or two Gaussians. The lattice constants (shown in the figures) and the variance σ_d of d are given in Å. The following values of σ_d have been calculated: (*a*) $X_{DMSO} = 0$, $\sigma_{d1} = 0.24$, $\sigma_{d2} = 1.57$; (*b*) $X_{DMSO} = 0.05$, $\sigma_{d1} = 0.25$, $\sigma_{d2} = 1.92$; (*c*) $X_{DMSO} = 0.3$, d = 4.15 Å, $\sigma_{d1} = 0.85$, $\sigma_{d2} = 4.29$; (*d*) $X_{DMSO} = 0.8$, d = 4.19 Å, $\sigma_{d1} = 0.62$, $\sigma_{d2} = 2.36$; (*e*) $X_{DMSO} = 1$, $\sigma_d = 0.2$.

wide-angle diffraction are already observed at $X_{DMSO} = 0.3$. At the concentration $X_{DMSO} = 0.3$ the "shoulder" disappears and the sharp peak becomes wider, and it is accompanied by a very diffuse peak below it (Fig. 6 *c*). The diffraction peak cannot be fitted by one Gaussian function. One needs two Gaussian functions to be able to do it. This means that the hexagonal lattice has become quite disordered. The orthorombic lattice that was observed at small X_{DMSO} transfers to the hexagonal one.

Fig. 6, *c* and *d*, shows that with a further increase in X_{DMSO}, up to $X_{DMSO} = 0.8$, membrane structure (including the tilt angle) does not change qualitatively. However, it is clearly seen that the diffraction pattern becomes less diffuse.

DMSO is adsorbed on the membrane surface and already at small volume concentrations affects its structure and intermembrane interactions. Moreover, it penetrates into the polar part of membrane. This was shown directly in neutron diffraction experiments (Balagurov and Gordeliy, 1986; Vasilenko et al., 1988).

At $X_{DMSO} \approx 0.9$ the diffraction pattern is drastically changed. The diffraction pattern (Fig. 6 *e*) with a single narrow diffraction peak relating to the interdigitated phase was described earlier (Ranck et al., 1977; McIntosh et al., 1983).

Thus the experimental data and physics of DMSO/water mixture result in the following conclusion: DMSO is adsorbed on the membrane surface, presumably displacing water molecules and affecting its properties and intermembrane interactions. Moreover, DMSO apparently forms with the polar surface of membrane a network of hydrogen bonds that stabilizes it and serves as an extra barrier to the membrane's transition to the liquid (L_α) phase, which causes an increase in the temperature of phase transition (gel $\rightarrow L_\alpha$).

Solvation or entropic nature of short-range intermembrane repulsive forces

The intermembrane distance is reduced with the increase in DMSO concentration at small X_{DMSO}. This fact was used to analyze the balance of intermembrane interactions in the presence of DMSO (Yu and Quinn, 1995).

It seems that intermembrane distance does not change considerably at higher concentrations of DMSO, except the concentrations at which the membranes are in interdigitated phase (Figs. 1 and 5). Unfortunately, one cannot make a definite conclusion about the precise value of bilayer thickness from the electron density profiles (Fig. 5). It has been mentioned that DMSO can modify the profiles (see above). In any case, the reduction of the intermembrane distance at small concentrations of DMSO has been proved. It means an unusual behavior: for other solvents, like, for example, glycerol, d_w changes in the opposite way, at least at small solvent concentrations in water.

The balance of forces is determined by short-range repulsive forces (Rand and Parsegian, 1989; Israelachvili and Wennerström, 1992):

$$P_H = P_0 \exp[-d_w/\lambda], \tag{2}$$

and the van der Waals attractive forces:

$$P_W = H\left(\frac{1}{d_w^3} - \frac{2}{d^3} + \frac{1}{(d + d_w)^3}\right), \tag{3}$$

where λ is the decay length of solvation forces and H is the Hamaker constant (Parsegian and Ninham, 1970; Israelachvili, 1991) equal to

$$H \approx \frac{3kT}{4}\left(\frac{\epsilon_m - \epsilon_s}{\epsilon_m + \epsilon_s}\right)^2 + \frac{3h\nu_e}{16\sqrt{2}}\frac{(n_m^2 - n_s^2)^2}{(n_m^2 + n_s^2)^{3/2}} \tag{4}$$

To analyze the dependence of van der Waals forces on X_{DMSO} in the whole range of DMSO concentrations, we used Eq. 4 for the Hamaker constant, dielectric constant $\epsilon_m = 2$, and retractive index $n_m = 1.45$ for the membranes (McIntosh et al., 1989). ϵ_s and n_s for different mixtures (different X_{DMSO}) were taken from the papers by Lindberg and Kenttämaa (1960) and Cowie and Toporowski (1961).

The Hamaker constant reduces sharply from $1.6kT$ at $X_{DMSO} = 0$ to $\sim 0.8kT$ at $X_{DMSO} = 0.2$. With a further increase in X_{DMSO} it changes slowly, reaching a minimum of $0.65kT$ at $X_{DMSO} \approx 0.5$, and increases only up to $\sim 0.7kT$ at higher concentrations.

This means that short-range repulsive forces in the presence of DMSO have to change considerably to lead to the experimentally observed behavior of intermembrane distance.

There are two different hypotheses about the origin of the repulsive forces. The first one is based on the assumption that a disturbance of solvent structure (e.g., polarization) by polar membrane surface is sufficient to result in the experimentally observed forces (Rand and Parsegian, 1989). According to this hypothesis, P_0 is determined by the magnitude of the disturbance of the solvent (e.g., polarization) at the membrane surface. The decay length λ can depend on the solvent (McIntosh et al., 1989) and the in-plane structure of membrane surface (Kornyshev and Leikin, 1989). Experiments with some nonwater solvents showed that the decay length increases with the growth of the size of solvent molecule (McIntosh et al., 1989). It was also shown that λ is smaller for an in-plane ordered surface (Kornyshev and Leikin, 1989). Supposing the decay length of solvation forces to be dependent on the volume properties of the solvent (Rand and Parsegian, 1989; McIntosh et al., 1989), one could conclude that in the presence of DMSO it can only increase, as the volume of the DMSO molecule is greater than that of water (McIntosh et al., 1989). From this it would follow that the equilibrated intermembrane distance should be bigger in the presence of DMSO than in the case of pure water. But this is in conflict with the experimental data.

Consequently, one should assume that it is the changes in the properties of membrane surface in the presence of DMSO that are responsible for the invariability of the

equilibrated intermembrane distance. In the framework of the "polarization" mechanism of the origin of repulsive forces, it could be justified by a high packing ordering of the polar groups of lipid molecules in the membrane plane (Kornyshev and Leikin, 1989). At small concentrations of DMSO, however, wide-angle diffraction peaks are almost the same (Yu and Quinn, 1995, and the previous section of this paper), and wide-angle diffraction did not show any extra reflexes in the range $d = 6 \div 12$ Å, which could have indicated ordering of polar heads. Thus, to account for the reduction of intermembrane distance, one has to assume a considerable decrease in magnitude of hydration pressure P_0 (e.g., solvent polarization) at the membrane surface in the presence of DMSO.

Another possible explanation could be based on the hypothesis of the entropic nature of "hydration" forces (Israelachvili and Wennerström, 1992), according to which short-range intermembrane repulsive forces arise from thermal fluctuations of lipid molecules along the normal of the membrane plane. However, the experimental data described here cannot themselves complete the analysis of the forces under discussion. Nevertheless, it is clear that DMSO modifies the membrane interface and, it seems, provides a new possibility in the study of short-range repulsive forces between membranes. An accurate study of the dependence of intermembrane distance on DMSO concentration as well as direct measurements of the forces and the polarization of membrane interface would be of great interest.

A possible biological account of the DMSO influence on the structure of lipid bilayers

The interaction between DMSO and polar membrane surface, as seen from the wide-angle diffraction picture, affected the close packing of hydrocarbon chains of lipid molecules. This apparently gives rise to defects in the bilayer structure, which must result in its increasing permeability. Indeed, it is of common knowledge that in many cases the biological and therapeutic impact of DMSO result from its ability to serve as vehicle, carrying drugs into the cell. It is related to the fact that DMSO increases membrane permeability (Wood and Wood, 1975).

Moreover, Yu and Quinn's data (1995) and our data indicate that DMSO strongly affects the structure and interaction of membranes already at concentrations of several percent. It also agrees well with the concentration dependence of the DMSO biological and therapeutic impact (Wood and Wood, 1975).

It seems that even in small proportions DMSO causes dehydration of the membrane surface. It drives water out of the membrane surface and intermembrane space through changes in intermembrane interaction. Apparently there is only a strongly bonded solvent near the membrane surface that brings down the freezing temperature (Gleeson et al., 1994).

VIG is very grateful to the Alexander von Humboldt Foundation and Prof. G. Büldt for the support of this work. The authors also sincerely thank Prof. I. A. Vasilenko for his help with the materials.

REFERENCES

Balagurov, A. M., and V. I. Gordeliy. 1986. Influence of ethylenglycol and dimethylsulfoxide on egg lecithin membranes. *Joint Inst. Nucl. Res. Commun. (Dubna).* P14–86-644:1–8.

Blaurock, A. E. 1982. Evidence of bilayer structure and of membrane interactions from x-ray diffraction analysis. *Biochim. Biophys. Acta.* 650:167–207

Blaurock, A. E., and C. A. Worthington. 1966. Treatment of low angle x-ray data from planar and concentric multilayered structures. *Biophys. J.* 6:305–312.

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

Cevc, G., and D. Marsh. 1985. Hydration of noncharged lipid bilayer membranes. Theory and experiments with phosphatidylethanolamines. *Biophys. J.* 47:21–31.

Cevc, G., and D. Marsh. 1987. Phospholipid Bilayers. John Wiley and Sons, New York. 442.

Cowie, J. M. G., and P. M. Toporowski. 1961. Association in the binary liquid system dimethyl sulfoxide-water. *Can. J. Chem.* 39:2240–2243.

Doucet, M. Y., F. Calmes-Perault, and M. T. Durand. 1965. La constante dielectrique du dimethylsulfoxide pur et de ses mélanges aqueux. *C. R. Acad. Sci. Paris.* 260:1878–1881.

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

Gennis, B. R. 1989. Biomembranes. Springer-Verlag, London. 533.

Gleeson, J. T., S. Erramilli, and S. M. Gruner. 1994. Freezing and melting water in lamellar structures. *Biophys. J.* 67:706–712.

Gordeliy, V. I. 1996. Possibility of direct experimental check-up of the theory of repulsion forces between amphiphilic surfaces via neutron and x-ray diffraction. *Langmuir.* 12:3498–3502.

Gordeliy, V. I., V. G. Cherezov, A. V. Anikin, M. V. Anikin, V. V. Chupin, and J. Teixeira. 1996a. Evidence of entropic contribution to "hydration" forces between membranes. *Prog. Colloid Polym. Sci.* 100:338–344.

Gordeliy, V. I., V. G. Cherezov, and J. Teixeira. 1996b. Evidence of entropic contribution to "hydration" forces between membranes. Part II. Temperature dependence of the "hydration" force: a small-angle neutron scattering study. *J. Mol. Struct.* 383:117–124.

Gordeliy, V. I., L. V. Golubchikova, A. Kuklin, A. G. Syrykh, and A. Watts. 1993. The study of single biological and model membranes via small-angle neutron scattering. *Prog. Colloid Polym. Sci.* 93:252–256.

Gordeliy, V. I., V. I. Luzhikov, A. G. Syrykh, and V. G. Cherezov. 1994. Application of the x-ray diffractometer DRON to the study of long period structures. *Joint Inst. Nucl. Res. Commun. (Dubna).* 14–91-388: 1–11.

Guggenheim, E. A. 1952. Mixtures. Clarendon Press, Oxford.

Hagelman, R. F., T. C. Evans, and E. F. Riley. 1970. Modification of radiation effect on eye by topical application of dimethyl sulfoxide. *Radiat. Res.* 44:368–342.

Honger, T., K. Mortensen, J. H. Ipsen, J. Lemmich, R. Bauer, and O. G. Mouritsen. 1994. Anomalous swelling of multilamellar lipid bilayers in the transition region by renormalization of curvature elasticity. *Phys. Rev. Lett.* 74:3911–3915.

Inoko, Y., and T. Mitsui. 1978. Structural parameters of dipalmitoyl phosphatidylcholine lamellar phases and bilayer phase transitions. *J. Phys. Soc. Jpn.* 44:1918–1924.

Israelachvili, J. N. 1991. Intermolecular and Surface Forces. Academic Press, London.

Israelachvili, J. N., and H. Wennerström. 1992. Entropic forces between amphiphilic surfaces in liquids. *J. Phys. Chem.* 96:520–531.

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.

Kim, J. T., J. Mattai, and G. G. Shipley. 1987. Bilayer interactions of ether- and ester-linked phospholipids: dihexadecyl- and dipalmitoylphosphatidylcholines. *Biochemistry.* 26:6599–6603.

Kirchner, S., and G. Cevc 1993. Temperature variation of lipid membrane structure and the hydration force in fluid lamellar phase: experimental studies with dimyristoylphosphatidylcholine multibilayers. *Europhys. Lett.* 23:229–235.

Kornyshev, A. A., and S. Leikin. 1989. Fluctuation theory of hydration forces: the dramatic effects of inhomogeneous boundary conditions. *Phys. Rev. A.* 40:6431–6438.

Le Neveu, D. M., R. P. Rand, V. A. Parsegian, and D. Gingell. 1977. Measurements and modification of forces between lecithin bilayers. *Biophys. J.* 18:209–230.

Lindberg, J. J., and J. Kenttämaa. 1960. Some considerations of the structure of dimethyl sulfoxide-water mixtures in the light of thermodynamic and dielectric behaviour. *Suom. Kemistilehti.* B33:104–107.

Lowelock, J. E., and M. W. H. Bishop. 1979. Prevention of freezing damage to living cells by dimethyl sulphoxide. *Nature.* 183:1394–1395.

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

McIntosh, T. J., S. Advan, R. E. Burton, D. V. Zhelev, D. Needham, and S. Simon. 1995. Experimental test for protrusion and undulation pressures in phospholipid bilayers. *Biochemistry.* 34:8520–8532.

McIntosh, T. J., R. V. Daniel, and S. A. Simon. 1983. Induction of an interdigitated gel phase in fully hydrated phosphotidylcholine bilayers. *Biochim. Biophys. Acta.* 731:109–114.

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

Pande, C., R. Callender, R. Henderson, and A. Pande. 1989. Purple membrane: color, crystallinity, and the effect of dimethyl sulfoxide. *Biochemistry.* 28:5971–5978.

Parsegian, V. A., and B. W. Ninham. 1970. Temperature-dependent van der Waals forces. *Biophys. J.* 10:664–674.

Pascher, I., S. Sandell, and H. Hauser. 1981. Glycerol conformation and molecular packing of membrane lipids. *J. Mol. Biol.* 153:807–824.

Ranck, J. L., T. Keira, and V. Luzzati. 1977. A novel packing of the hydrocarbon chains in lipids. The low temperature phases of dipalmitoyl phosphatidylglycerol. *Biochim. Biophys. Acta.* 488:432–441.

Rand, R. P., and V. A. Parsegian. 1989. Hydration forces. *Biochim. Biophys. Acta.* 988:351–376.

Schipley, G. G. 1973. Recent x-ray diffraction studies of biological membranes and membrane components. *In* Biological Membranes, Vol. 2. D. Chapman and D. Wallach, editors. Academic Press, London. 1–89.

Simon, S. A., S. Advani, and T. J. McIntosh. 1995. Temperature dependence of the repulsive pressure between phospholipid bilayers. *Biophys. J.* 69:1473–1483.

Simon, S. A., and T. J. McIntosh. 1984. Interdigitated hydrocarbon chain packing causes the biphasic transition behaviour in lipid/alcohol suspensions. *Biochim. Biophys. Acta.* 773:169–172.

Simon, S. A., T. J. McIntosh, and A. D. Magid. 1988. Magnitude and range of the hydration pressure between lecithin bilayers as a function of headgroup density. *J. Colloid Interface Sci.* 126:74–83.

Soper, A. K., and A. Luzar. 1992. A neutron diffraction study of dimethyl sulfoxide-water mixtures. *J. Chem. Phys.* 97:1320–1331.

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

Tchoubar, D., F. Rousseau, C. H. Pons, and M. Lemonier. 1978. Small angle scattering setting at LURE: descriptions and results. *Nucl. Instrum. Methods.* 152:301–305.

Tommila, E., and A. Pajunen. 1969. The dielectric constants and surface tensions of dimethyl sulfoxide-water mixtures. *Suom. Kemistilehti B.* 41:172–176.

Vasilenko, I. A., V. I. Gordeliy, L. A. Tonkonog, and V. L. Borovyagin. 1988. Structure arrangement of phospholipid membranes in non-water polar solvents. *Biol. Membr.* 514:428–438.

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

Wood, D. C., and J. Wood. 1975. Pharmacologic and biochemical considerations of dimethyl sulfoxide. *Ann. N.Y. Acad. Sci.* 243:7–19.

Worthington, C. R. 1969. The interpretation of low-angle x-ray data from planar and concentric multilayered structures. *Biophys. J.* 9:222–234.

Worthington, C. R., G. I. King, and T. J. McIntosh. 1973. Direct structure determination of multilayered membrane-type systems which contain fluid layers. *Biophys. J.* 13:480–494.

Yi, P. N., and R. C. MacDonald. 1973. Temperature dependence of optical properties of aqueous dispersions of phosphatidylcholine. *Chem. Phys. Lipids.* 11:114–134.

Yu, Z.-W., and P. J. Quinn. 1995. Phase stability of phosphatidylcholines in dimethylsulfoxide solutions. *Biophys. J.* 69:1456–1463.

Zaccai, G., V. I. Gordeliy, and A. Watts, editors. 1994. Membrane structure, dynamics, and interactions via neutron and x-ray scattering. *Biophys. Chem.* 49:1–100.