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Preferential interaction of dimethyl sulfoxide and phosphatidyl choline membranes

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Abstract

The interaction free energy of dimethyl sulfoxide (DMSO) and two types phospholipid membranes has been assessed from measurements of vapor pressure. The lipids were phosphatidyl cholines with respectively (14:0/14:0) (DMPC) and (16:0/18:1) (POPC) fatty acid chains. The results were expressed in terms of the iso-osmolal preferential interaction parameter, $\Gamma_{\mu 1}$, which remained negative under all experimental conditions investigated here. This shows that water–membrane interactions are more favorable than DMSO–membrane interactions. This condition is known as preferential exclusion of DMSO (or preferential hydration of the membrane), and implies that the local (interfacial) concentration of the solute is reduced compared to the bulk. At room temperature and 1 *m* DMSO, $\Gamma_{\mu 1}$ was -0.3 to -0.4 for both lipids. This corresponds to a sizable reduction in the DMSO concentration in a zone including at least the first two hydration layers of the membrane. Possible origins of the preferential exclusion are discussed.

As a direct consequence of the pronounced preferential exclusion, DMSO generates an osmotic stress at the membrane interface. This tends to stabilize lipid phases of low surface areas and to withdraw water from multilamellar stacks of membranes. Based on this, we suggest that the preferential exclusion of DMSO explains both the modulation of phase behavior and the constriction of multilamellar aggregates induced by this solute.

Keywords: Vapor pressure; Me_2SO; Membrane–solute interaction; Preferential exclusion; Osmotic stress; Thermodynamics

1. Introduction

Dimethyl sulfoxide (DMSO) has been applied in diverse purposes for biotechnology. Some examples include use in permeabilization and fusion of cell membranes and its widespread use as a protecting agent in freezing protocols [1–3]. The molecular origin of these (and other) effects of DMSO on living cells remains to be fully elucidated, but many pieces of evidence have pointed toward membrane–DMSO interactions and the concomitant perturbation of membrane properties as a basic factor. If indeed so, phospholipid bilayer membranes in mixed water–DMSO solvents are perhaps the most promising model system for studies of mechanisms underlying the biological effects of this compound. An important example of this type of work is the X-ray scattering studies by Yu and Quinn [4–7]. They found that multilamellar stacks of lipid membranes showed a pronounced reduction in the repeat distance upon addition of DMSO, and this effect has also been observed in other studies [8,9]. Another characteristic effect of DMSO on lipid membranes, which has been reported in several works, is its modulation of lipid phase behavior. For example, it increases the gel ($P_{\beta'}$) to lamellar (L_α) phase transition temperature, T_m, for a variety of phospholipids, but decreases the temperature, T_H, for the L_α to hexagonal (H_{II}) phase transition in phosphatidyl ethanolamine lipids [3,4,6,8–10]. Interestingly, in some cases, these effects of DMSO have been shown to be opposite to those exerted by other small solutes of related chemical structure such as acetone and small alcohols [10,12–14]. DMSO has also been shown to significantly affect non-equilibrium processes such as the leakage rate of a fluorescent probe from phospholipid liposomes [2,15]. The molecular interpretation of these observations is still debated. Quinn and coworkers have proposed that DMSO dehydrates the membrane interface by partitioning into this zone and hence displacing water [6]. Anchordoguy et al. [2,15], on the other hand,

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rationalized their results by suggesting a strongly temperature-dependent penetration of DMSO into the hydrophobic core of the membrane.

In the current work, we address this problem through a thermodynamic analysis of the membrane–solvent interface. More specifically, we report preferential interaction parameters for DMSO and two types of phosphatidyl choline membranes. This approach has its roots in multicomponent solution thermodynamics [16] and over the last decades it has been specialized and sophisticated for the description of proteins in mixed solvents [17–20]. The framework of preferential interactions involves both stringent thermodynamic relationships, such as the linkage between the concentration of a small solute and its effect on macromolecular equilibria [21] and suggestions for the structural interpretation and modeling of experimental data [18,19]. Following its success in the description of protein solutions, preferential interaction theory has also been used in discussions of membrane–solute interactions [15,22–27]. Direct experimental determinations of preferential interaction parameters for membrane systems, however, remain sparse [28,29][1].

2. Methods and materials

The lipids dimyristoyl phosphatidyl choline (DMPC) and palmitoyl oleyl phosphatidyl choline (POPC) (both >99%) were purchased as powders from Avanti Polar Lipids (Alabaster, AL) and used as supplied. DMSO (>99.9%) was from Fluka (Buchs, Switzerland) and water was freshly prepared from a Milli-Q purification system (Millipore, Bedford, MA).

The free energy of membrane–DMSO interactions was derived from measurements of vapor pressure conducted in a specially designed instrument built in this laboratory [34]. The equipment measures the pressure difference, ΔP, between a cell and a reference under controlled conditions of temperature and composition. The experimental set-up allows independent changes of these two parameters without changing the sample or breaking the vacuum. In the current application, this was done so that the concentration of DMSO, m_3, (in moles DMSO per kg water)[2] was always the same in the cell and reference. Below, we sketch out the experimental procedures, but we note first that the general experimental principle is that under these conditions of equal m_3, ΔP will quantify the effect (in terms of total vapor pressure) of solvent–membrane interactions.

The lipids were hydrated in excess of pure water (ca. 5% w/w lipid) for several hours at room temperature (POPC) or 40 °C (DMPC) during which time they were repeatedly exposed to vigorous shaking. The lipid suspension was extruded to 100 nm unilamellar vesicles using the equipment from Lipex (Northern Lipids Inc. Vancouver, BC), and the lipid concentration in the product was determined gravimetrically to within $\pm 0.02\%$ (w/w) as described previously [28]. About 1 ml unilamellar vesicle suspension was weighed (± 0.01 mg) in the cell of the vapor pressure equipment and mounted onto the equipment. Approximately the same amount of pure water was weighed in the reference and mounted. Atmospheric air was then removed from the cell and reference by repeatedly allowing the gas above the liquid to expand into an evacuated 500 ml flask (see Ref. [34]). This was continued until the vapor evacuated from the flask was free of non-condensable components (when passed through two N_2(l) freeze traps). After calculating the (small) loss of water associated with the out-gassing procedure, the amount of water was adjusted to be equivalent in cell and reference.

All parts of the vapor pressure equipment, except the cell and reference, are housed in an air-bath at 90 °C. Liquid DMSO in a 100 ml flask mounted directly on the vapor pressure equipment (at 90 °C) is allowed to evaporate into a previously evacuated container to a pressure (determined to within ± 3 μbar) of about 16 mbar. Subsequently, DMSO vapor is condensed into the cell (or reference) kept at 0 °C, and the amount of solute transferred is quantified from the pressure reduction in the container. This procedure of ''gas phase titration'' enables precise control of the DMSO concentration, and an experimental series involves a number of measurements where the cell-to-reference pressure difference (ΔP) is recorded. Throughout the series, various temperatures and DMSO concentrations are assessed, always keeping m_3 equal in the cell and reference.

DMSO turned out to be more difficult to handle than both more [35] and less [28] volatile compounds, which have previously been investigated. The intermediate vapor pressure of DMSO (about 1 mbar at room temperature) required longer equilibration times probably due to the slow evaporation of DMSO condensed on the sides of the cell during gas phase titration. To facilitate equilibration (signified by a constant ΔP reading) we adopted the following procedure. After gas phase titration with DMSO, the temperature in the cell/reference thermostat was increased to 45 °C, and the system was allowed to equilibrate overnight. After recording ΔP (45 °C) the thermostat temperature was lowered to 26 °C and subsequently to 5 °C with several hours of equilibration at each temperature. The temperature was then raised to 45 °C again to confirm the earlier ΔP (45 °C) reading. In cases where this did not match the first 45 °C measurement, the cycle of the three temperatures was repeated. After reading ΔP, the sample

[1] We note that so-called osmotic stress measurements, which have been extensively utilized in studies of membrane hydration (see Ref. [30] for a review), rely on the same theoretical foundation as preferential interactions [31–33]. Osmotic stress measurements generally utilize large hydrophilic solutes which are assumed to be completely excluded from the interfacial zone. Thus, osmotic effect of full exclusion rather than the degree of preferential interaction is assessed.

[2] Throughout this work we will use the conventional notation of subscripts 1, 2 and 3 for, respectively water, lipid and solute (DMSO).

and reference were out-gassed as described above to ensure the absence of any contamination of air. Out-gassing gives rise to the loss of both water and DMSO from the sample. However, since the gas phase in equilibrium with, e.g. 1 *m* aqueous DMSO contains about 99.99% (mol/mol) water [36], the loss of DMSO during out-gassing was neglected in the data analysis.

Each lipid sample was gas phase titrated with five to seven aliquots of DMSO, and this covered the 0.2–2 *m* concentration range.

3. Results and data analysis

Fig. 1 illustrates measured ΔP values plotted against the molal concentration of DMSO, m_3. Panel A represents an experiment where a 5.16% (w/w) suspension of 100 nm unilamellar POPC vesicles was titrated with DMSO to a final concentration of about 2 *m*. Panel B shows an analogous experiment with DMPC (6.46% w/w). It appears that the slope of ΔP vs. m_3 is negative for both lipids. In as much as the vapor is practically pure water and m_3 is equal in the cell and reference, the negative slopes show that the membrane interacts more favorably with water than with DMSO. This condition is referred to as preferential hydration of the membrane or preferential exclusion of DMSO from the membrane interface. To quantify this, we need to turn from the total vapor pressures illustrated in Fig. 1 to partial pressures, which directly specify the chemical potential of a component. This can be stringently done in a number of ways [37–40], but for the current system, it is adequate to simply equate the total vapor pressure to the partial pressure of water. This simplification is justified by vapor pressure data for binary aqueous DMSO [36], which shows that the partial pressures of DMSO in the concentration range investigated here is well below the experimental uncertainty specified as the scatter in Fig. 1.

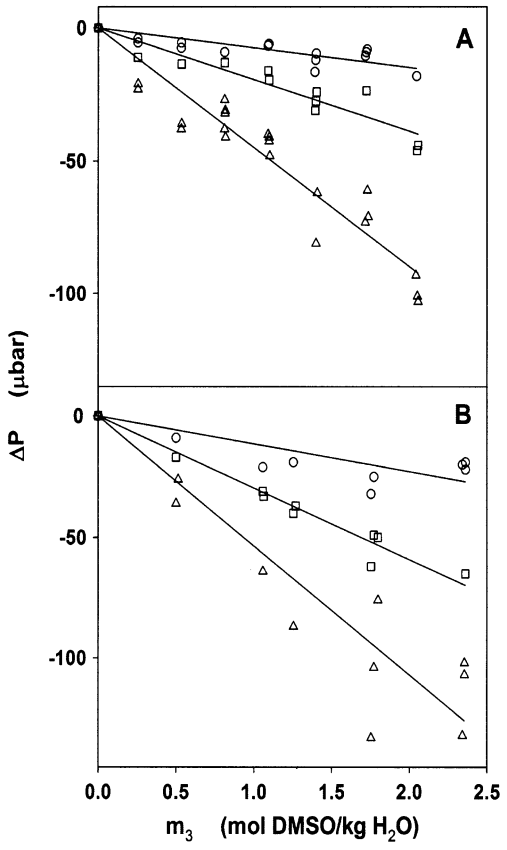

Fig. 1. Raw data from the vapor pressure measurements showing the difference in vapor pressure, ΔP, between a cell containing water + DMSO + lipid and a reference with water + DMSO. The DMSO concentration, m_3, in moles per kg H_2O was equal in the cell and reference, and ΔP thus reflects the effect on the vapor pressure of lipid–solvent interactions. Negative values signify that the vapor pressure in the cell is lower than in the reference. The graph illustrates results from trials (see Methods and materials) where, respectively, 5.16% (w/w) POPC (panel A) and 6.46% (w/w) DMPC (panel B) were titrated with DMSO. The experimental temperature is indicated by circles (5 °C), squares (26 °C) and triangles (45 °C).

To proceed, we calculate the iso-osmolal preferential binding parameter, $\Gamma_{\mu 1}$, which is defined [33]:

$$\Gamma_{\mu 1} \equiv (\partial m_3/\partial m_2)_{T,P,\mu 1} \quad (1)$$

In Eq. (1), *m* denotes molal concentrations and *T*, *P* and μ are, respectively temperature, pressure and chemical potential. Again, subscripts 1, 2 and 3 signify, respectively water, lipid and DMSO. A comprehensive analysis of $\Gamma_{\mu 1}$ and its relation to the general multicomponent solution thermodynamics is available elsewhere [33,41]. Here, we only emphasize the immediate meaning of the definition in Eq. (1), i.e. that $\Gamma_{\mu 1}$ signifies the (positive or negative) number of DMSO molecules needed to balance out the change in the chemical potential of water brought about by the addition of one phospholipid molecule. It follows that negative values of $\Gamma_{\mu 1}$ signify that the membrane has a higher affinity for water than for DMSO and vice versa. To numerically estimate the derivative in Eq. (1), we introduce the quantity Δm_3, which is the difference in DMSO-concentration between the reference solution and a phospholipid (cell) sample with the same water activity. Δm_3 is specified by the pressure difference, ΔP, and the composition dependence of the vapor pressure dP_{ref}/dm_3. Hence

$$\Delta m_3 \cong \frac{\Delta P}{(dP_{ref}/dm_3)} \quad (2)$$

where P_{ref} is the water vapor pressure in the reference taken from Lai et al. [36]. If it is assumed that the cell-reference difference in lipid concentration is small, the derivative in Eq. (1) can be replaced with the ratio of concentration changes and combined with Eq. (2).

$$\Gamma_{\mu 1} \cong \frac{\Delta P}{m_2(dP_{ref}/dm_3)} \quad (3)$$

The approximations in Eqs. (2) and (3) are valid for low m_2 and m_3. Based on vapor pressure binary aqueous DMSO

[36] and the absence of a systematic dependence of $\Gamma_{\mu 1}$ on m_2 found in this work, it is concluded that the errors of assuming low m_2 and m_3 are unimportant in comparison to the experimental scatter. A similar conclusion has been reached for other systems [28,33].

Fig. 2 shows $\Gamma_{\mu 1}$ calculated according to Eq. (3) from the data in Fig. 1, and plotted as a function of the DMSO concentration, m_3. It appears that $\Gamma_{\mu 1}$ decreases approximately linearly with m_3 for both lipids. The slope of linear fits to the data at 26 and 45 °C fall in the -0.3 to -0.4 m^{-1} range. Specific values of $\Gamma_{\mu 1}/m_3$ along with 95% confidence limits of the fitted slopes are given in the legend of Fig. 2. At 5 °C the experimental scatter becomes rather large for both lipids. This probably reflects the low total vapor pressure at this temperature which inevitably makes $\Gamma_{\mu 1}$ more sensitive to experimental error (c.f. Eq. (3)). In addition, slow equilibration of gradients including concentration imbalances across the membranes (which in the case of DMPC are in the gel phase at this temperature), may bring about additional scatter. In any case, the experimental precision at 5 °C only allows a rather coarse estimate of $\Gamma_{\mu 1}$ at this temperature.

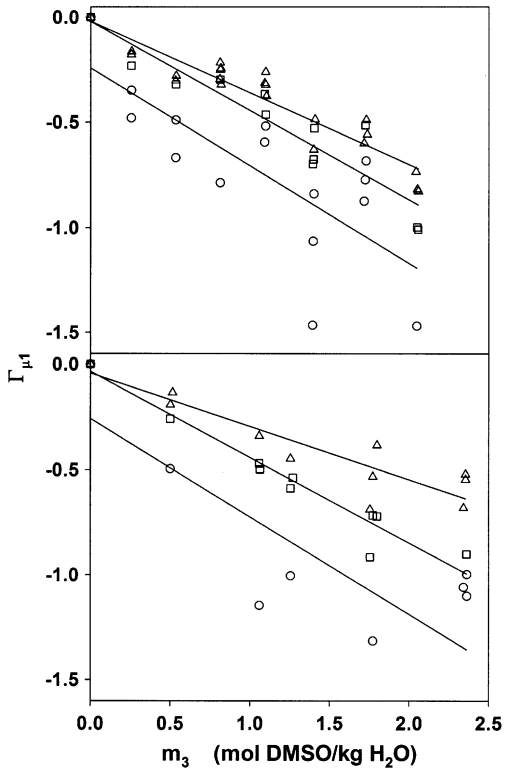

Fig. 2. The iso-osmotic preferential interaction parameter, $\Gamma_{\mu 1}$, calculated from the data in Fig. 1 according to Eq. (3) and plotted as a function of the concentration of DMSO, m_3. All symbols have the same meaning as in Fig. 1. For POPC (panel A) the slopes of the linear fits are, respectively -0.46 m^{-1} [-0.70, -0.23 m^{-1}], -0.42 m^{-1} [-0.54, -0.30 m^{-1}] and -0.34 m^{-1} [-0.40, -0.26 m^{-1}] for 5, 26 and 45 °C. The intervals in square brackets indicate 95% confidence limits for the fitted slopes. For DMPC (panel B) the slopes at the three temperatures are -0.50 m^{-1} [-0.17, -0.84 m^{-1}], -0.41 m^{-1} [-0.34, -0.47 m^{-1}] and -0.29 m^{-1} [-0.37, -0.21 m^{-1}].

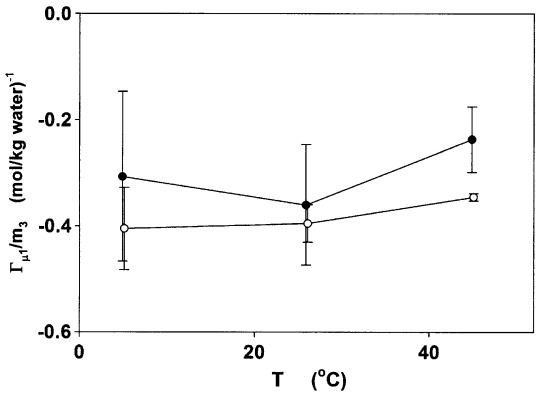

Fig. 3. Average values of, $\Gamma_{\mu 1}/m_3$ determined from graphs similar to Fig. 2 for a total of five titration trials (two for POPC, open symbols; and three for DMPC, filled symbols) plotted as a function of the temperature. Error bars identify S.E.

Slopes from plots similar to Fig. 2 were compiled and plotted as a function of temperature in Fig. 3. While the average $\Gamma_{\mu 1}/m_3$ values are consistently smaller (less negative) for DMPC than for POPC the difference is not statistically significant. Also, the experimental precision is not high enough to allow a significant determination of the temperature dependence of $\Gamma_{\mu 1}/m_3$.

4. Discussion

Although practically absent from the membrane interior, polar (and ionic) solutes distinctively modulate the properties of lipid bilayers. Such modulations have been ascribed to so-called Hofmeister mechanisms [22,42], i.e. indirect effects of the solute induced through changes in the solvent properties. This was recently discussed by Feng et al. [43], who noted that "the significance of direct interactions between co-solutes and phospholipid molecules independent of their effect on water structure has yet to be assessed". Below we attempt to address this by discussing some aspects of the extent, origin and effect of preferential interactions of PC membranes suspended in aqueous DMSO.

4.1. Extent of preferential exclusion

Although only few quantitative reports on membrane–solute preferential interactions are available for comparison, it seems that the degree of preferential exclusion of DMSO is rather high. Thus, the exclusion of glycerol from DMPC [28] membranes, expressed as $\Gamma_{\mu 1}/m_3$, is less than half the value found for DMSO here, and our preliminary work on the disaccharide trehalose [29] suggests that even this larger and more hydrophilic solute is less excluded from the membrane interface than DMSO. Possible origins and con-

sequences of this observation, which may appear surprising for a compound with two hydrophobic methyl groups, are discussed below. First, we analyze the degree of preferential exclusion through the application of the so-called bulk-local domain model, which rationalizes the preferential binding parameter in terms of a concentration imbalance between the bulk and a local domain (i.e. a zone near the biomolecule interface). This structural interpretation of the thermodynamic data was formally described by Record et al. [18], although earlier works had used a similar analysis of protein conformations in mixed solvents [44,45]. One of the key results derived from the bulk-local domain model is the relationship

$$\Gamma_{\mu 1} \approx B_3 - m_3 B_1 / m_1 \quad (4)$$

where B_3 and B_1 are the number of, respectively, solute (DMSO) and water molecules in the local domain. The specific size of these parameters obviously relies on the extent of the local domain, which must be determined from other (non-thermodynamic) information. Eq. (4) is formally correct when m_3 is the solute concentration in the bulk domain and the left-hand side is $\Gamma_{\mu 1,\mu 3}$—i.e. the preferential binding parameter measured by dialysis equilibrium. However, we have previously argued that $\Gamma_{\mu 1,\mu 3} \approx \Gamma_{\mu 1}$ for lipid systems in excess water [28]. If we consider the case $B_3 = 0$ corresponding to complete exclusion of DMSO from the local domain, insertion in Eq. (4) suggests that $B_1 \approx 20$ (note that $m_1 = 55.5$ mol/kg). Consequently, the data can be accounted for by a local domain of about 20 water molecules per lipid which are free of any DMSO. While this illustrates the (average) extent of exclusion of DMSO, we hasten to stress that it is most likely not a realistic picture of the interface. Thus, the permeability (albeit moderate) of DMSO documents its presence near and inside biological membranes [46–48]. It follows that $B_3 > 0$ and thus that $B_1 > 20$. While the specific magnitude of the local domain cannot be determined here, we note that it must extend well beyond a water monolayer covering the lateral area of the membrane (about 60 $Å^2$—or six water molecules—per lipid). Monolayer coverage of the rugged solvent accessible area of a hydrated PC membrane corresponds to approximately 13 water molecules per lipid [49]. This is also below the minimal value of B_1 derived from Eq. (4). Even if B_1 is set to 30, which corresponds to the amount of water per lipid separating fully hydrated multilamellar PC membranes [30], the local concentration of DMSO (Eq. (4)), is significantly lowered (approx. three times) with respect to the bulk. We conclude that the degree of preferential exclusion observed here involves a pronounced reduction in the local DMSO concentration in a zone involving at least two hydration layers.

Unfortunately, relationships between the extent of preferential exclusion of DMSO and the temperature, lipid phase and fatty acid unsaturation cannot be determined unambiguously from the current data—although the results at 45 °C (Fig. 3) seem to hint at a stronger exclusion for POPC than for DMPC.

4.2. Effect of DMSO on membrane dimensions and phase behavior

One of the well-documented effects of DMSO on lipid bilayers is its ability to stabilize the gel ($P_{\beta'}$) phase with respect to fluid (L_α) phase. Thus, the main (gel-to-fluid) transition temperature, T_m, of different types of phospholipids has been shown to increase with the concentration of DMSO in the solvent [3,6]. In addition, DMSO has been found to decrease the temperature (T_h) of the transition from L_α to the hexagonal (H_{II}) phase in phosphatidyl ethanolamines [6]. These solute-induced effects are paralleled by carbohydrates and cosmotropic ions, and for these latter groups of solutes, it is generally believed that this reflects their preferential exclusion and the concomitant osmotic stress and stabilization of conformations of low interface area [22–25,27,42,50]. Accordingly, we suggest that the effect of DMSO on membrane phase behavior is governed by its preferential exclusion. More specifically, preferential exclusion gives rise to an increase in the osmotic pressure of the bulk over that of the interfacial region, resulting in a movement of water away from the interfacial zone (down the osmotic gradient). This leads to tighter packing of the lipid molecules and favoring of membrane processes involving the release of water to the bulk. Thus, due to its lower surface area, the gel ($P_{\beta'}$) phase is stabilized over the fluid (L_α) phase and T_m increases. This conclusion is in line with the suggestion recently put forward by Kinoshita et al. [10,11]. These authors ascribed their diffraction and fluorescence data to "an increase in the interaction free energy of membrane surface segments and the solvent upon increasing DMSO concentrations". This conclusion is tantamount to the negative $\Gamma_{\mu 1}$ reported here. In fact, the effect can readily be quantified since the transfer free energy, ΔG_{trans}, of moving lipid from water to aqueous DMSO can be calculated from the $\Gamma_{\mu 1}$ data (see Ref. [28]). At room temperature and $m_3 = 1$ *m*, we find $\Delta G_{trans} = 0.9 \pm 0.1$ kJ/mol. It follows that the unfavorable effect hypothesized by Kinoshita et al. involves an increase in the free energy by about 1 kJ/mol lipid when the DMSO concentration is increased from 0 to 1 *m*. The current observations are also in line with IR-spectroscopic studies on reversed micelles by Yu et al. [51]. The latter work concluded that DMSO interacts more favorably with water than with the micelle interface, again signifying its preferential exclusion form the interfacial zone.

Several studies have shown that DMSO acts to decrease the repeat distance in stacks of multilamellar membranes [5,6,8,9]. This has been suggested to involve shrinking of the intermembrane space, and it may reach a level where very little water appears to remain there [6]. The origin of this effect, which is opposite to that of chemically related compounds such as acetone [10], has been widely dis-

cussed. The current results suggest that this may simply be an effect of the osmotic stress induced by the exclusion of DMSO. At 40% (w/w) DMSO for example, where the repeat spacing of PC membrane stacks shrinks to values less than that of lyophilized multilayers, full exclusion of DMSO from the intermembrane space translates into an osmotic stress of about 300 atm [36]. This is sufficient to generate a substantial reduction in the intermembrane spacing of stacked bilayers [30].

4.3. Origins of preferential exclusion

The substantial preferential exclusion of DMSO may seem puzzling considering its two methyl groups. Other amphiphilic compounds of comparable size exhibits a moderate propensity to partition into the membrane [52]. Such partitioning generates a positive contribution to $\Gamma_{\mu 1}$ and tends to decrease T_m in contrast to the behavior of DMSO. This disparity between structurally related solutes emphasizes the question of what mechanisms underlie the preferential exclusion of DMSO. While this topic remains to be specifically addressed, two points may be interesting to note. First, a number of recent theoretical works have identified steric hindrance as a major cause for preferential exclusion of solutes from protein interfaces [53–56]. Secondly, many reports have suggested molecular clustering in "simple" (binary) aqueous DMSO [57–61]. The (short-lived) clusters may consist of either DMSO molecules held by dipolar interactions of the SO-groups or hydrogen bonded SO-water adducts. The most recent attestation of this comes from the diffraction studies of Koga et al. [57], who found evidence for the former type of aggregate in dilute DMSO solutions. Combining these two lines of information leads to the suggestion that the exclusion of DMSO could rely on steric hindrance of small clusters, which more than balances out a possible hydrophobic attraction for the membrane due to the methyl groups.

In conclusion we have found that DMSO is preferentially excluded from the interface of two types of phosphatidyl choline membranes. This implies that the membranes interacts more favorably with water than with DMSO, and hence that the interfacial zone is enriched with water compared to the bulk. The extent of this effect is substantial and involves a partial depletion of DMSO in at least two hydration layers. The preferential exclusion of DMSO and the concomitant osmotic stress may explain the effects of this solute on both membrane phase behavior and dimensions of multilamellar membrane stacks.

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