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Solvation effects of dimethyl sulphoxide on the structure of phospholipid bilayers

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Abstract

The effect of dimethylsulphoxide (DMSO), a widely used solvent in life sciences to stabilise biological membrane structures at low temperatures, on the structure of model membranes has been examined using X-ray diffraction methods. It was found that increasing concentrations of DMSO in water caused a progressive decrease in lamellar repeat spacings of multilamellar dispersions of both saturated and mono-unsaturated phosphatidylcholines. The lamellar repeat spacings were temperature-dependent but with dispersions in excess 40 wt.% DMSO, repeat spacings were less than that of the phospholipids in the dry state. One dimensional electron density profiles of the lipid bilayers were calculated and the thickness of the phosphatidylcholine bilayers were determined. It was inferred from the data that the thickness of liquid-crystal bilayers decreases in the presence of DMSO and that the DMSO molecules penetrate between the polar head groups of the phosphatidylcholines, resulting in an increase in area occupied by phospholipid at the bilayer surface. © 1997 Elsevier Science B.V.

Keywords: X-ray diffraction; DMSO; Phosphatidylcholine; Bilayer structure; Phospholipid hydration; Biomembranes

1. Introduction

Dimethylsulphoxide (DMSO) is a water-miscible solvent that has wide applications in cell biology [1]. The cryoprotective property of DMSO was discovered more than 35 yrs ago by Lovelock and Bishop [2], and concentrations ranging up to 40 wt.% are commonly employed in long-term cryopreservation of a variety of cells, tissues, and organs [3]. The ability of DMSO to induce fusion between cells [4] and liposomes [5], to increase solute permeability across cell barriers [6] and membranes [7], to induce cellular differentiation [8], and to protect cells against the harmful effects of ionising radiation [9] are other well documented actions of the solvent. The molecular mechanisms by which DMSO exerts its effects are presently unknown but many are explained by modulation of membrane structure or stability.

Phosphatidylcholines, when dispersed in excess water, are known to form one of four types of

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lamellar phases; subgel, gel, ripple and liquid-crystal [10]. These structures provide a useful model to investigate the properties of phospholipids in biological membranes. The present study was undertaken to examine the effect of DMSO on the structure of model phospholipid membranes using synchrotron X-ray diffraction methods in order to understand the molecular mechanisms of action on biological membranes. Our previous studies of dispersions of phospholipids in aqueous DMSO solutions showed that the solvent has a marked effect on the phase behaviour of these phospholipids and, in particular, caused the disappearance of the ripple phase of saturated phosphatidylcholines, resulting in a direct lamellar-gel to liquid-crystal phase transition [11], and the destabilisation of the lamellar liquid-crystal phase of unsaturated phosphatidylethanolamine [12]. We report here that increasing concentrations of DMSO in water caused a progressive decrease in lamellar repeat spacings of saturated and mono-unsaturated phosphatidylcholines to values well below that observed by the phospholipids in the dry state. Based on calculated electron density profiles across the bilayer, we conclude that thickness of liquid-crystal bilayers decreases in the presence of DMSO, which penetrates between the polar head groups of the phospholipid molecules resulting in an increase in area occupied by phospholipids at the bilayer surface.

2. Materials and methods

Lipid dispersions with a lipid:solvent ratio of 1:2 (wt./wt.) were prepared in aqueous DMSO solutions. Dispersions of dipalmitoylphosphatidylcholine (DPPC) used to determine X-ray diffraction parameters in the liquid-crystal phase contained lipid:solvent ratios of 1:0.25 and 1:0.7 for water and 35 wt.% DMSO solution, respectively. Homogeneous dispersions were prepared by subjecting the samples to several freeze–thaw cycles between about −20°C and above the respective main phase transition temperatures interspersed with vigorous vortex mixing. Synchrotron X-ray diffraction measurements were performed on 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 [13]. Changes in X-ray scattering intensity profiles were recorded during heating and cooling scans of 5°/min.

The method to calculate the one-dimension electron density distribution across the lipid bilayer structures has been described previously [11,14]. Briefly, integrated intensities $I(h)$ for a range of diffraction orders (h) were obtained from low-angle scattering intensity profiles. And electron density profiles, in arbitrary units, were expressed as [14]: $\rho(x) = \sum g(h)|F(h)|\cos(2\pi hx/d)$, where $F(h)$ is the structure factor and equals to the root of $I(h)$ in amplitude, $g(h)$ the phase of $F(h)$ of the hth order diffraction and d the repeat spacing of the multibilayers.

3. Results and discussion

The high intensity of a synchrotron X-ray source has been exploited to relate d-spacings of phosphatidylcholine dispersions in excess aqueous DMSO solutions to temperature during heating scans at 5°/min through the gel to liquid-crystal phase transition. Fig. 1 shows lamellar repeat spacings of DPPC as a function of temperature over the range 20° to 100°C. Also shown are d-spacings of DPPC freeze-dried from an aqueous dispersion. It can be seen that increasing concentrations of DMSO in water causes

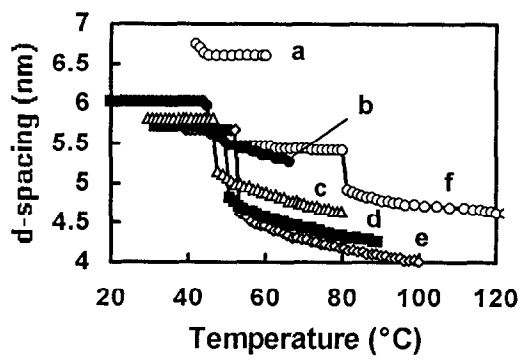

Fig. 1. Temperature dependence of lamellar repeat spacings of DPPC dispersed in excess aqueous DMSO solutions recorded during a heating scan at 5°/min. (a) ○, 0 wt.%; (b) ●, 40 wt.%; (c) △, 60 wt.%; (d) ■, 80 wt.%; (e) ◇, 100 wt.%; and (f) ○, freeze-dried phospholipid (dihydrate).

a progressive increase in temperature of the transition from gel to liquid-crystal phase in DPPC, which is characterised by an abrupt decrease in lamellar repeat spacing. This change in d-spacing coincides with a change in the wide-angle diffraction pattern which provides information about the packing arrangement of the hydrocarbon chains and is characteristic for each of the phases of phosphatidylcholine [10]. Thus, a sharp peak centered at 0.42 nm, characteristic of gel phase transforms into a diffuse peak centered at 0.42 nm typical of liquid-crystal phase at higher temperatures (data not shown). The temperature of this transition in the freeze-dried DPPC (80°C) indicates that the phospholipid is in the form of a dihydrate [15]. Most interestingly, DMSO caused a decrease in the lamellar repeat spacing of the phospholipid in the gel and particularly in the liquid-crystal phase; concentrations of DMSO greater than 40 wt.% cause shrinkage to values considerably less than that of the freeze-dried phospholipid.

To check whether the effects of DMSO are similar in unsaturated phospholipid, the relationship between lamellar repeat spacings and temperature in dispersions of dioleoylphoshatidylcholine (DOPC) in 52 wt.% and pure DMSO was compared with dispersions in excess water. The results are presented in Fig. 2. The data in this case are complicated due to freeze-thawing of solvent, but the phase assignment can be made by reference to the scattering from the acyl chain packing recorded at wide angles. The

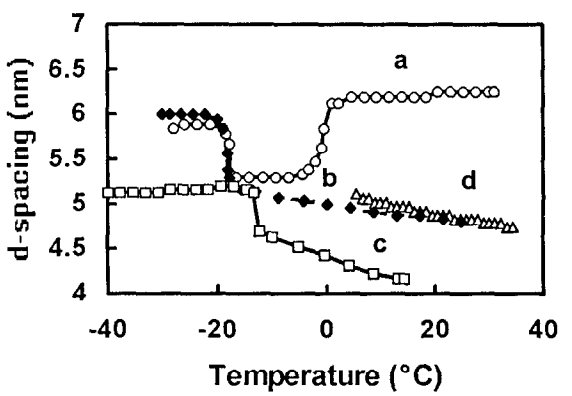

Fig. 2. Temperature-dependence of lamellar repeat spacings of DOPC dispersed in (a) ○, excess water; 5; (b) ◆, 52 wt.% DMSO; (c) □, DMSO; (d) △, freeze-dried DOPC recorded during a heating scan at 5°C/min.

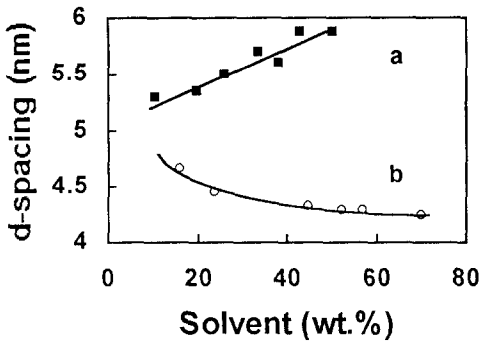

Fig. 3. Relationship between lamellar d-spacing and weight percentage of water ■, (a) or DMSO: ○, (b) added to freeze-dried DPPC. The spacings were measured at 55°C for water and 65°C for DMSO.

relatively low value of repeat spacing of the gel phase of DOPC in water compared to that in DMSO solutions, for example, is due to removal of water from the lattice as bulk water freezes; the subsequent expansion at about 0°C results when ice melts and water penetrates into the lattice again. In the fluid phase, however, the lamellar repeat distance of the liquid-crystal phospholipid clearly decreases with increasing DMSO concentration. At a concentration of about 50 wt.% of DMSO in water the lamellar repeat spacing is approximately the same as the freeze-dried phospholipid and, as in the case of DPPC, much less than this when dispersed in excess pure DMSO. Estimates of the thickness of the solvent layer in fully hydrated multibilayer dispersions of phosphatidylcholines derived from X-ray data give values of about 1.5 nm in the fluid phase [16]. The lamellar repeat spacings of dispersions of phosphatidylcholines in solvent containing more than 50 wt.% DMSO decrease by much more than this value, indicating a thinning of the lipid bilayer.

The dramatic effect of DMSO on lamellar repeat spacing of DPPC is further illustrated in Fig. 3. This shows d-spacing as a function of percentage weight of solvent added to freeze-dried preparations of phospholipid at temperatures where the acyl chains are in a disordered state. The measurements in DMSO were recorded at 65°C rather than at 55°C as the mixture with DMSO does not form a liquid-crystal phase at the lower temperature at low solvent:lipid ratios. Water causes an expansion of the lattice

which is known to be associated with a slight decrease in bilayer thickness as the polar groups are solvated [17]. Addition of DMSO, by contrast, results in a shrinkage of the lamellar repeat spacing, which may be accompanied by a thinning of the bilayer.

To examine this effect, electron density distributions through the repeat structure of DPPC bilayers formed by dispersions of the phospholipid in water and 35 wt.% DMSO were calculated. These profiles are shown in Fig. 4 together with consensus molecular structures of the smectic mesophases in gel (a and b) and liquid-crystal states (c and d). The phases used in the electron density calculations were $-+-$ for DPPC dispersions in water at gel state and $-+-$ for all the others [11,18]. Sharp wide-angled reflections centered at a spacing of 0.42 nm with a shoulder on the high-angle side in both samples examined at 25°C confirmed that the lipids were in a gel phase with the hydrocarbon chains tilted at an angle of about 27° to the bilayer normal [18]. The apparent fluid layer thickness, presented by the peak-to-peak distance of the electron density profile, is reduced from 2.23 nm to 1.69 nm (compare curves a and b in Fig. 4) by replacing water with 35 wt.% DMSO solution, presumably due to a decrease in repulsive force acting across the bilayer-solvent interface [11,17]. At the same time, there is an apparent increase in thickness of the bilayer of about 0.22 nm. Because the wide-angle reflections remain unchanged, a change in the angle of tilt of the hydrocarbon chains cannot be responsible for the increased thickness. It could be due to either a reorientation of the phosphocholine residue from a more or less parallel [19] towards a perpendicular alignment with respect to the bilayer plane, or a preferential location of the relatively electron-dense sulphur atoms of DMSO at the bilayer-solvent interface, or a combination of both effects.

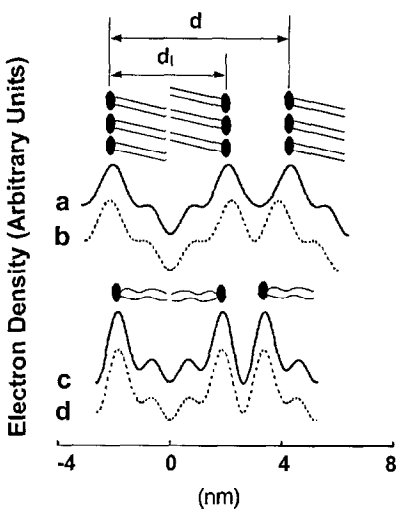

Fig. 4. Electron density profiles of DPPC dispersions in water (————) and 35 wt.% DMSO solution (---) in gel state at 25°C (a and b) and liquid-crystal state at 55°C (c and d). Dispersions were prepared in ratios of lipid:solvent (wt:wt) of 1:2 (a & b), 1:0.25 (c), 1:0.7 (d). The most probable arrangement of the phospholipid molecules in the structures are shown with the filled circles representing the polar groups and the lines depicting the hydrocarbon chains.

The repeat spacing (5.26 and 5.21 nm) and apparent bilayer thickness (3.79 and 3.70 nm) of the lipid dispersions in the liquid-crystal phase (Fig. 4c and 4d) are almost identical for DPPC in water (lipid:solvent = 1:0.25) and 35 wt% DMSO (lipid:solvent = 1:0.7). This means that despite the presence of more than twice as much solvent in the sample dispersed in aqueous DMSO, the dimensions of the putative solvent layer are the same in both dispersions. One explanation consistent with the data is that DMSO contributes to that part of the electron density profile designated as phospholipid bilayer as already concluded in the case of lipid in the gel phase. Using the method of Lis et al. [17] and taking a value of specific density of 35 wt.% DMSO solution of 1.02, a bilayer thickness of 3.16 nm is obtained. Considering a value of 3.70 nm for the apparent lipid thickness obtained from calculated electron density across the bilayer repeat, location of the electron-dense sulphur atoms in the region of the phosphate groups of the phospholipid is inferred. This would result in an expansion of the area occupied by the phospholipid molecules at the bilayer surface and a corresponding decrease in thickness. It is also apparent that although the oil–water partition coefficient of DMSO (0.003) [20] is greater than water in phospholipid bilayers (< 0.0001) [21], extensive penetration of DMSO into the hydrocarbon domain, as suggested by Anchordoguy et al. [7], is not supported by the electron density calculations.

These results suggest that the action of DMSO in increasing passive permeability of membranes is due,

in part, to a reduction in the diffusion pathway of solutes moving from one side of the membrane to the other. Additional effects that cannot be quantified from the present data could be to enhance the partition coefficient of solutes between the aqueous phase and the membrane and/or to potentiate the molecular mechanisms of diffusion of solutes within the membrane lipid bilayer matrix.

The thickness of the lipid matrix in a membrane is important not only for its function in forming a permeation barrier. Recent studies of the secretory pathways of proteins in eukaryotic cells has led to the hypothesis that Golgi enzymes and plasma membrane proteins may be sorted by matching their different hydrophobic segments with the thickness of different lipid domains within the Golgi bodies [22]. Studies of the effect of chain length of phospholipids on the activity and molecular dynamics of reconstituted Ca-ATPase from skeletal sarcoplasmic reticulum has shown that acyl chain length of 18 carbons gave the optimum enzymic activity [23]. This observation also strongly supports the importance of the hydrophobic span in modulating the functional activity of intrinsic proteins and suggests that DMSO may exert many of its actions on living cells by altering the thickness of the hydrocarbon domain of membranes.

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