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Molecular Basis for Dimethylsulfoxide (DMSO) Action on Lipid Membranes

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Dimethylsulfoxide (DMSO) is an aprotic solvent that has the ability to induce cell fusion and cell differentiation and enhance the permeability of lipid membranes.[1,2] It is also an effective cryoprotectant.[3,4] Insights into how this molecule modulates membrane structure and function would be invaluable toward regulating the above processes and for developing chemical means for enhancing or hindering the absorption of biologically active molecules, in particular into or via the skin.[5] We show here by means of molecular simulations that DMSO can induce water pores in dipalmitoyl−phosphatidylcholine (DPPC) bilayers and propose this to be a possible pathway for the enhancement of penetration of active molecules through lipid membranes. DMSO also causes the membrane to become floppier, which would enhance permeability, facilitate membrane fusion, and enable the cell membrane to accommodate osmotic and mechanical stresses during cryopreservation.

DMSO may be considered as a small amphiphile. Such molecules tend to be rapidly incorporated at the lipid-water interface of membranes. They influence many membrane processes and appear to act in a nonspecific manner; that is, their effect is not due to any interaction with a specific molecule. An important application of DMSO is in topical or transdermal drug delivery, where its powerful ability to increase skin permeability is exploited.[6,7] How DMSO (or many of the other known penetration enhancers) increase the skin permeability is still a mystery, but a greater understanding of its mechanism of action in this respect would be invaluable for the rational design of molecules that modulate the transport of active molecules in membranes. Molecules that *decrease* the permeability of membranes (or skin) are also of considerable interest for their potential ability to block absorption of toxic chemicals such as insect repellents and pesticides and herbicides.

DMSO's ability to modulate membrane permeability may also contribute to its cryoprotectant activity. Since DMSO can readily permeate into the cell interior, its primary role in cryopreservation is perceived to be the prevention of intracellular crystallization of ice.[4] In the "slow cooling" cryopreservation protocol,[4] ice forms in the extracellular space causing concentration of solute in the residual water. This sets up an osmotic imbalance between the inside and outside of the cell. The rate of cooling must be sufficiently slow to enable solutes and water to exchange across the cell membrane so as to keep the osmotic imbalance to a minimum. On thawing, the ice melts making the extracellular space hypotonic relative to the inside of the cell, which can give rise to the possibility of rapid ingress of water resulting in cell lysis. Enhanced permeability of the membrane to solutes would allow for rapid but controlled osmotic equilibration between the intra and extracellular space. The DMSO concentrations employed in cryopreservation vary widely from ∼1 to 13 mol %.[4]

We have carried out molecular dynamics (MD) simulations of the interaction of DMSO molecules with a DPPC lipid bilayer using a coarse-grained approach, wherein a number of atoms are represented by a single particle. Coarse graining is computationally efficient enabling longer time and length scales to be accessed within the simulations. The DMSO molecule was represented by a dimer with specific interaction parameters. All other simulation and force-field parameters were identical to those employed by Marrink et al.[8] Simulations were carried out for a series of concentrations (0−45 mol % with respect to H_2O) of DMSO in the DPPC system under anisotropic NPT conditions at 323 K and 1 bar up to simulations times of 0.8 μs using GROMACS.[9] The bilayer was comprised of 512 DPPC molecules in water. We also calculated the mechanical properties of the membrane, namely the area compressibility modulus K_A and bending rigidity κ, as a function of DMSO concentration. The interaction parameters, methodology, and results are detailed in Supporting Information.

The simulations reveal a number of features that are significant in respect to the effects of DMSO on membrane structure and function. Thus DMSO molecules readily partition into the bilayer occupying a position just beneath the lipid headgroups, reduce bilayer thickness, increase headgroup area, markedly reduce both the area compressibility modulus and the bending rigidity of the membrane, and induce water pore formation. With regards to DMSO distribution, bilayer thickness, and headgroup area, the current results are consistent with both experimental data[10,11] and recent atomistic simulations.[12] The results, however, are not entirely consistent with an earlier set of simulations[13] possibly because of the short time scales (2ns) accessed in that study. The area compressibility modulus K_A decreased from 330 ± 20 for the pure DPPC system to 90 ± 10 mN/m for the 12 mol % DMSO system. The bending modulus κ decreased from 5.6×10^{-21} (pure DPPC system) to 1.1×10^{-21} J for the bilayer with 12 mol % DMSO. It was not possible to determine these quantities for the higher concentrations of DMSO as these membrane systems very rapidly formed pores.

The reduction in the area compressibility modulus and the bending rigidity arise from DMSO's rather specific positioning within the bilayer. The DMSO molecules tend to reside just below the headgroups of the lipid molecules. By doing so, the DMSO molecules push the lipid heads apart and hence act as spacers at the headgroup level, so that the average headgroup area per molecule increases. As a consequence the tail region of the membrane also becomes less dense, which enables the tails of the neighboring lipids to expand into an effectively larger volume. The overall effect is that DMSO causes the bilayer to become "floppier" and hence readily amenable to bending.

The remarkable event of the formation of a water pore was observed for the 27 mol % DMSO system after 240 ns (Figure 1). The formation of the pore was initiated by a fluctuation in the

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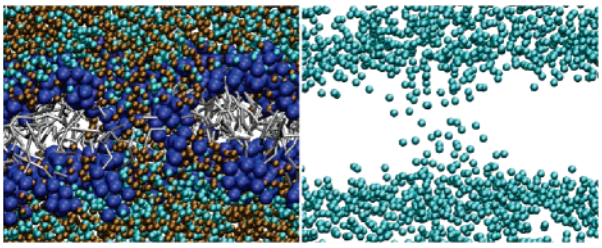

Figure 1. Water pore formation in a tensionless DPPC bilayer with 27 mol % DMSO after 261.4 ns. Water molecules are shown in cyan, DMSO in brown, DPPC headgroup and glycerol backbone particles in blue, and hydrocarbon particles in light gray.

bilayer that caused two of the lipid molecules sitting on the opposite sides of the bilayer to get deeper into the bilayer. Water molecules then started to enter the bilayer (most likely encouraged by the headgroups of these lipids), which were followed by DMSO molecules that in turn facilitated the entry of more water into the bilayer until there was a continuous chain of water. At this point the lipids reoriented to form an hourglasslike pore that rapidly expanded to a size that was stable for the remainder of the simulation.

Theoretical ideas of pore formation in membranes are dominated by classical nucleation theory. The model assumes the membrane to be a two-dimensional elastic medium with a hole. The medium is characterized by a free energy per unit area γ (the surface tension), while the hole edge is characterized by a free energy per unit length or line tension Γ. The stability of a hole of radius r is given by the interplay between the positive, edge free-energy that is proportional to the pore perimeter and the loss in the surface-area free-energy (which is proportional to the area of the hole) due to the formation of the hole, that is, $\Delta G = \Gamma 2\pi r - \gamma \pi r^2$. The model yields an activation barrier of $G^{\#} = \pi \Gamma^2/\gamma$ and predicts that pores with a radius below a certain critical value are unstable while those above this radius will grow indefinitely until the membrane ruptures. Further refinements of the model incorporate curvature energy in addition to the surface tension,[14] the effect of fluctuating undulations, and the entropy associated with pore shape.[15] The refinement of Tolpekina et al.[14] suggests that there is a local minimum just past the critical radius above which the free energy increases with increase in pore size and hence explains the formation of metastable pores. Our results indicate that DMSO does indeed affect the key physical properties of the membrane that favor pore formation, namely the lowering of the bending and tensile moduli that make the membrane readily amenable (low energy cost) to forming curved surfaces.

Pore formation in itself is not remarkable. The significance here is that DMSO induces and stabilizes a pore in a system that is tensionless. Pores can be induced in skin membranes in experiments by applying mechanical stress[16] or an electric field.[17] Pores have also been observed in MD simulations of phospholipid bilayers as transient structures in tensionless bilayers[18] and when the membrane is subjected to either an electric field (electroporation)[18] or mechanical stress.[14,19] We propose that the observed DMSO-induced water pore formation could be an important possible mechanism by which DMSO enhances the permeability of membranes. This mechanism is consistent with the experimentally observed concentration dependent effects of DMSO on membranes. Experimentally, permeability enhancement only occurs at high DMSO concentrations (>26 mol %),[6] and high concentrations are also required to induce pore formation in the simulations. Second, DMSO is known to enhance the penetration of both hydrophilic and hydrophobic molecules,[6] and the enhancement of *hydrophilic* compounds by DMSO has always been difficult to explain. Molecular mechanisms proposed to explain permeability enhancement by DMSO include interaction with membrane proteins leading to structural defects at the protein−lipid interface (DMSO readily denatures proteins and is known to alter the conformation of the intercellular keratin in the stratum corneum),[20] modulation of the membrane structure to make it more fluid, and a "solvent effect" that facilitates biologically active molecule partitioning from the formulation into the skin.[6] These mechanisms can explain the enhancement of hydrophobic molecules but not (convincingly) of hydrophilic molecules. In fact it is difficult to conceive of a simple mechanism for the enhancement of a hydrophilic penetrant other than by the proposed formation of water pores.

In conclusion, we have provided a molecular basis for the action of DMSO in membrane fusion processes, cryopreservation, and membrane permeability enhancement. DMSO makes the membrane significantly floppy, which would facilitate membrane fusion processes, enable cells to more readily accommodate stresses in cryopreservation protocols, reduce the barrier to molecular transport, and assist pore formation. The DMSO molecules appear to reside just below the headgroup region and act as spacers/pivots that enhance lipid−lipid separation, enabling the bilayer to readily adopt a curved form to accommodate any stress. This mechanism of action may be a general feature of small amphiphillic molecules including short chain alcohols. At high concentrations, DMSO has been observed to induce water pores in the membrane, which could be a possible mechanism of action for membrane permeability enhancement particularly to hydrophilic molecules.

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Supporting Information Available: Interaction parameters for DMSO, details of methodology, and further results. This material is available free of charge via the Internet at http://pubs.acs.org.

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