Ion Transport through Chemically Induced Pores in Protein-Free Phospholipid Membranes

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We address the possibility of being able to induce the trafficking of salt ions and other solutes across cell membranes without the use of specific protein-based transporters or pumps. On the basis of realistic atomic-scale molecular dynamics simulations, we demonstrate that transmembrane ionic leakage can be initiated by chemical means, in this instance through addition of dimethyl sulfoxide (DMSO), a solvent widely used in cell biology. Our results provide compelling evidence that the small amphiphilic solute DMSO is able to induce transient defects (water pores) in membranes and to promote a subsequent diffusive pore-mediated transport of salt ions. The findings are consistent with available experimental data and offer a molecular-level explanation for the experimentally observed activities of DMSO solvent as an efficient penetration enhancer and a cryoprotectant, as well as an analgesic. Our findings suggest that transient pore formation by chemical means could emerge as an important general principle for therapeutics.

Introduction

Trafficking of ions through cell membranes, being coupled to energy transduction, is central to many cellular processes and functions.[1] A notable example is that of ion transport across cell membranes of neurons, which defines the signal (action potential) that is conveyed from one nerve cell to another. While the movement of ions across biological membranes is mainly mediated by specialized proteins, ions (as well as some small hydrophilic molecules) can leak in small amounts unassisted through transient defects[2] that include water pores.[3] Unassisted but *enhanced* transport of solutes through cell membranes is an important characteristic of many biomedical and biotechnological applications that include cryopreservation and drug and gene delivery. Clearly, in view of this prerequisite, it is highly desirable if one could induce, at will and in a controlled manner, transient defects such as pores in biological membranes.

Dimethyl sulfoxide (DMSO), a small amphiphilic molecule, seems to offer a potential means for achieving the above objective. It is widely employed in cell biology[4] as an effective penetration enhancer,[5] cryoprotectant,[6] and cell fusogen[7] and has been promoted clinically as a local analgesic[8] and a protective agent against ischemic injury.[9] Recent computational studies[10,11] suggest that the experimentally observed ability of DMSO to enhance the permeability of cell membranes is most likely coupled to DMSO-induced water pores in membranes.

On the basis of realistic *in silico* experiments, we provide here compelling evidence that small amphiphilic solutes such as DMSO are able to *promote* pore-mediated transport of ions across protein-free phospholipid membranes. More specifically, formation of transient defects (pores) in the presence of DMSO and subsequent transmembrane ionic leakage through the DMSO-induced water pores are witnessed at atomic resolution on a nanosecond time scale. These observations explain for the first time some of the important pharmacological actions of DMSO, e.g., analgesia, protection against ischemic injury, and cryopreservation, that involve modulation or disruption of ion or water transport across a cell membrane. Transient pore formation by chemical means could emerge as an important general principle for therapeutics.

Methods

Atomic-scale molecular dynamics (MD) simulations of lipid bilayers comprising 128 dipalmitoyl-phosphatidylcholine (DPPC) lipids were performed in aqueous solution containing DMSO and monovalent salt (NaCl or KCl). Two concentrations of DMSO, 10 and 15 mol % (lipid-free basis), were considered; at each DMSO concentration, two independent simulations were performed with either NaCl or KCl at the concentration 0.35 M.

The united atom force-field of Berger et al.[12] was used for describing DPPC lipids. For DMSO we employed the force-field developed by Bordat et al.,[13] while water was modeled using the simple point charge (SPC) model.[14] For sodium and chloride ions, we employed the set of parameters developed by Straatsma and Berendsen[15] and supplied within the Gromacs force-field.[16] Potassium ions were modeled following Roux and Beglov.[17] The Lennard−Jones interactions were truncated at 1 nm. The electrostatic interactions were handled using the particle-mesh Ewald (PME) method.[18,19] The simulations were performed at constant pressure of 1 bar (semi-isotropic coupling) and at constant temperature of 350 K which is above the phase transition temperature of DPPC bilayers in DMSO/water at all considered DMSO concentrations.[20] The temperature and pressure were kept constant with the use of the Berendsen coupling scheme,[21] using couplings constants of 0.1 and 1.0 ps, respectively.

The initial configurations were prepared as follows. We started with a DPPC lipid bilayer solvated in 3655 water molecules, which was equilibrated for 50 ns at a temperature of 350 K. The resulting structure was then used for preparing the initial configurations for all DPPC/DMSO/salt and DPPC/salt systems. For the DPPC/salt systems, 20 cations and 20 anions were added to the water phase, replacing randomly

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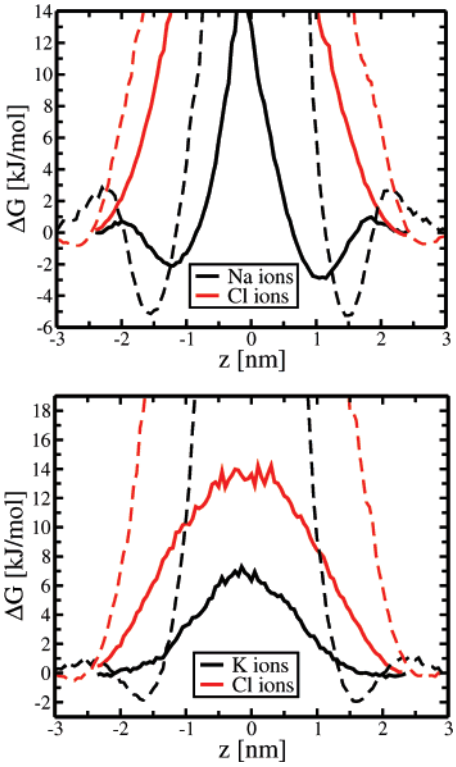

Figure 1. Estimated free energy profile $\Delta G(z)$ (potential of mean force) of ions as a function of the distance from the membrane center ($z = 0$) for membrane systems containing NaCl (top) and KCl (bottom). Solid lines correspond to systems with 10 mol % DMSO and dashed lines are for DMSO-free systems.

chosen water molecules. For the DPPC/DMSO/salt systems, all water molecules were removed from the box leaving the DPPC bilayer and the size of the box in the *z*-direction (the bilayer normal) was increased. This bilayer system was then solvated, first with DMSO and then with water, the total number of solvent molecules (DMSO and water) being set to 3655. Finally, randomly chosen water molecules were replaced by 20 cations and 20 anions.

The time step was 2 fs. Four bilayer systems with DMSO and salt (two concentrations of DMSO and two types of monovalent salt) were simulated for 100 ns each. As a reference, we also carried out two additional, 60-ns long simulations of DPPC membranes under influence of monovalent salt (NaCl or KCl) without DMSO. For the sake of comparison, two MD simulations of salt-free DPPC bilayers with 10 and 15 mol % of DMSO were taken from ref 11. The combined simulated time of all MD simulations amounted to 520 ns. All simulations were performed using the GROMACS suite.[16]

Results and Discussion

Because of the hydrophobic nature of its central core, a phospholipid bilayer under normal conditions is practically impermeable to ions. This reflects the prohibitively high free−energy barrier of the normal membrane to translocation of ions. An indication of the relative magnitude of this barrier to the various ions is illustrated for the DMSO-free and the DMSO-containing systems in Figure 1. The figure presents the potential of mean force (PMF), $\Delta G(z)$, which is the variation in free energy as a function of position along the membrane normal (the chosen reaction coordinate). The PMFs were estimated from the ion densities using $\Delta G(z) = k_B T \ln(\rho_{eq}/\rho(z))$, where ρ_{eq} is the average density of ions in bulk water and $\rho(z)$ is the average density of ions as a function of their position along the membrane normal ($z = 0$ corresponds to the membrane center). The PMFs are being used here only in a comparative way, as we are aware that the density distributions for the ions, which form the basis for the estimates, are far from being converged considering the so few ion leakage events. To accurately quantify the barriers, one would need to employ a more rigorous approach such as umbrella sampling.[23]

TABLE 1: Summary of DMSO-Induced Pore Formation and Pore-Mediated Ion Leakage

system	C_{DMSO} [mol %][a]	salt	T_{pore} [ns][b]	cations leaked	anions leaked
1	10.0	NaCl	> 100	0	0
2	10.0	KCl	50	21	13
3	15.0	NaCl	5	34	48
4	15.0	KCl	7	34	6

[a] Molar concentration of DMSO (lipid-free basis). [b] Time lapsed prior to pore formation. For salt-free bilayer systems containing 10.0 and 15.0 mol % DMSO, $T_{pore} = 20$ ns and $T_{pore} = 4$ ns, respectively.[11]

Addition of DMSO, at both concentrations 10 and 15 mol %, leads to considerable expansion and thinning of the DPPC membrane[11,22,24] coupled to formation of hydrophilic pores as observed previously[11] on the time scale of ∼20 ns for 10 mol % DMSO and ∼5 ns for 15 mol % DMSO concentration. Inclusion of salt ions into the systems reveals competition between the DMSO, which partitions into the lipid/water interface and acts as a spacer between the lipid headgroups to induce membrane expansion and water pores,[10,11,22] and the ions that appear to promote formation of tight complexes of the lipids, effectively stitching the membrane.[25−27] At 10 mol % DMSO, both competing factors appear to be subtly balanced; the Na ions (because of their relatively stronger binding with the phosphatidylcholine headgroups of the lipids) are able to overcome the effect of DMSO and prevent pore formation, while the moderately binding K ions are only able to retard pore formation. At 15 mol % DMSO, the effect of DMSO predominates and we observe pore formation for both salt ions. For easy comparison, the time taken for pore formation and number of leakage events (where applicable) for each of the systems studied are tabulated in Table 1.

At the low (10 mol %) DMSO concentration, the Na ions are able to prohibit pore formation (and consequently ion leakage) over the entire course of the simulation (100 ns). This is reflected by the permeation barriers for Na and Cl ions presented in Figure 1 (top). The presence of 10 mol % DMSO in a DPPC membrane with NaCl salt notably reduces the width of the barrier for permeation of Na ions (due to the DMSO-induced membrane thinning), but the height of the barrier is still too high to allow ion leakage to occur. In comparison, K ions (whose interaction with lipids is considerably weaker[27]) while unable to prevent pore formation do slow down the process, with pore formation now requiring up to 50 ns (versus 20 ns for a salt-free DPPC system with 10 mol % DMSO). It has to be emphasized that all the pore formation times mentioned above should be considered as suggestive rather than quantitative because of the small number of (although individually rather long) independent simulation runs. More reliable values for the characteristic times for pore formation would require a larger set of multiple MD runs each starting from a slightly different initial condition.

The formation of the transient water pores dramatically reduces the free energy barriers to permeation of ions through the membrane (Figure 1), so that ions are now able to leak across the membrane, avoiding highly unfavorable contacts with hydrocarbon chains in the membrane interior. For the bilayer

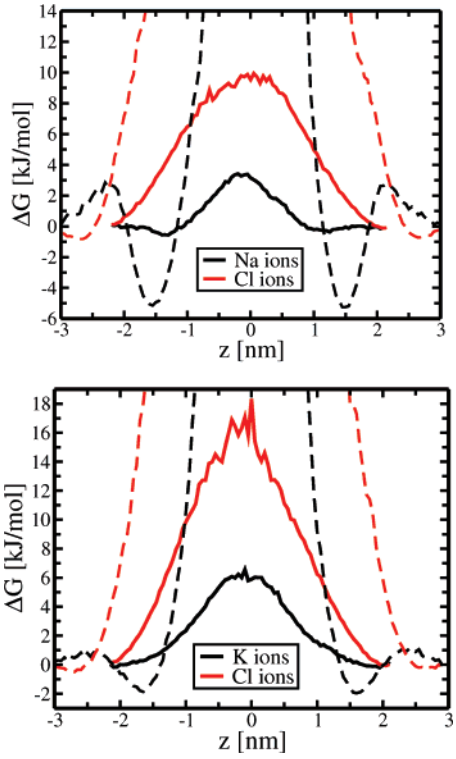

Figure 2. Estimated free energy profile $\Delta G(z)$ of ions as a function of the distance from the membrane center for systems containing NaCl (top) and KCl (bottom). Solid lines correspond to systems with 15 mol % DMSO and dashed lines are for DMSO-free systems.

system with 10 mol % DMSO containing KCl, where pore formation was observed, we noted 21 and 13 leakage events for K and Cl ions, respectively. The numbers of events seem commensurate with the free energy barriers for the K and Cl ions as given in Figure 1.

For the 15 mol % DMSO systems, we observe pore formation and associated ion leakage for both salts, NaCl and KCl. The free energy profiles for the various ions across these membranes are presented in Figure 2. The profiles for K and Cl ions indicate that the barrier for chloride leakage across the membrane is somewhat higher compared to what was seen for the system with 10 mol % DMSO, while the barrier for permeation of potassium ions is almost the same in both cases (cf. Figures 1 (bottom) and 2 (bottom)). Overall, for such a system containing NaCl, we witnessed 34 leakage events for Na ions and 48 leakage events for Cl ions over a period of ~90 ns. Remarkably, the total number of Na ions leaked is found to be smaller than that of Cl ions despite the lower barrier for permeation of Na ions as seen in Figure 2. This we believe is because of the strong interaction of sodium ions with lipid headgroups,[25,26] which considerably slows down the Na ion permeation[27,28] (this interaction of Na ions with the membrane's carbonyl region gives rise to the deep minima observed in $\Delta G(z)$ for the DMSO-free bilayer in Figure 2 (top)).

The general picture observed for the bilayer system with KCl and 15 mol % of DMSO is in many ways similar; the only difference is that potassium ions, being larger than sodium ions, interact only weakly with the lipid headgroups,[27] and the transport kinetics of the two ions, K and Cl, now more closely conform to the dictates of the respective free energy profiles (Figure 2). In all, we witnessed 34 leakage events for potassium ions and just 6 such events for chloride ions. Clearly, there seems to be a large difference in the barrier heights for chloride permeation (Figure 2) depending on whether the salt is NaCl

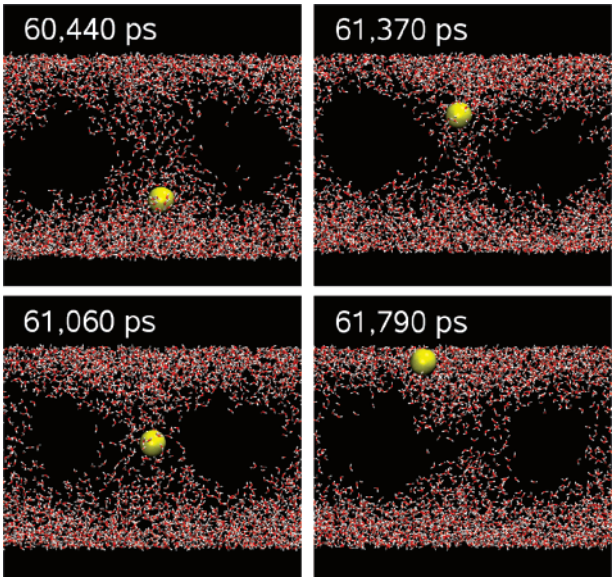

Figure 3. Permeation of a potassium ion (shown in yellow) through a transient water pore induced by the addition of 10 mol % of DMSO to a DPPC bilayer system. Water is shown in red and white; lipids, DMSO, and K and Cl ions are not shown.

or KCl, which naturally translates into the difference in numbers of permeation events for Cl ions, 48 vs 6. This probably results from a greater competition between the Cl and K ions for the water pore, relative to that between Cl and Na ions. With the NaCl salt, the stronger interaction of Na ions with the lipid headgroups is expected to limit the Na crossing events in favor of Cl crossings. Additionally, we note that the water pore size for the KCl-containing system was somewhat smaller than that for the NaCl system, which could be serving to increase the barrier to Cl ions.

From Table 1 we note that chloride leakage is considerably more pronounced at the lower DMSO concentration. The time for which the pore stays open for 10 mol % DMSO system is almost half of that of the 15 mol % DMSO system (because of the mentioned delay in pore formation) and yet the former system yields 13 Cl ion leakage events compared with only 6 events at the higher DMSO concentration. This disparity most likely results from DMSO molecules competing with Cl ions for permeation through the pore, with such competition being much greater in systems containing higher concentrations of DMSO molecules.

A series of simulation snapshots visualizing a typical leakage event of a potassium ion via a water pore for the 10 mol % DMSO system are shown in Figure 3. The permeation of ions across the membrane is a stochastic process driven mainly by thermal forces. In a certain sense the situation here differs from what has been observed for pore-mediated ionic leakage under influence of the transmembrane charge imbalance where electrostatic forces played a crucial role.[27,28] Because of its stochastic nature, each transmembrane ionic leakage was found to be accompanied by several unsuccessful (partly successful) permeation events. The characteristic time of ion leakage turns out to be highly sensitive to the type of ion; sodium ions cross the membrane considerably slower as compared to potassium and chloride ions. This is again because of the strong interaction of the Na ions with the carbonyl region of a DPPC membrane as well as with the lipid headgroups that form the "walls'' of hydrophilic pores.[25−28] As a result, a transmembrane ion permeation event which takes around 1 ns for K and Cl ions (Figure 3) can require an order of magnitude longer time in the

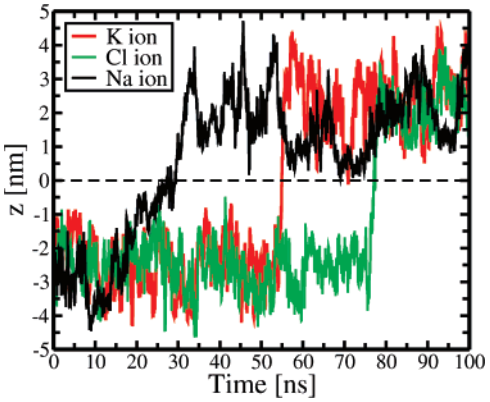

Figure 4. Typical MD trajectories of sodium (black line), chloride for the NaCl containing system (green line), and potassium (red line) ions leaked through transient water pores induced by the addition of 15 mol % of DMSO to a DPPC membrane. *z* denotes ion positions along the direction of the bilayer normal.

case of Na ions. Some representative trajectories for different types of leaked ions across the membrane (the *z*-direction) are shown in Figure 4: It is clearly seen that a Na ion can spend a rather long time inside the pore, a feature not generally observed for the K and Cl ions.

The observed effect of salt ions on pore formation is in line with experimental studies which show that the critical tension required to rupture a phospholipid membrane is higher in the presence of moderately binding potassium chloride as compared to poorly binding trimethylammonium chloride.[29] This finding indicates a direct inverse link between the propensity of membranes to form pores and the abilities of salt ions to bind to the membrane surface. Furthermore, a recent computational study of the tension-induced pore formation in phospholipid membranes in the presence of Na and Cl ions also suggests that salt ions can greatly reduce the stability of transient water pores: Sodium ions, being bound to the lipid/water interface, are found to increase the pore line tension, leading to a destabilization of the pore.[30]

In summary, we have carried out molecular dynamics simulations using realistic atomic-scale models and were able to directly observe diffusive transmembrane ionic leakage though transient water pores induced by DMSO. These results confirm that the water pores induced by DMSO can indeed promote permeability of hydrophilic solutes such as ions across phospholipid membranes. The findings explain the various experimentally observed pharmacological actions of dimethyl sulfoxide including penetration enhancement of hydrophilic molecules, and cryopreservation, where disruption or modulation of ion and/or water transport across a membrane is considered to be important. Finally, we note that there appears to be considerable interest as to whether other small molecules are able to induce water pores and promote ion leakage in membranes. The small chain alcohols, including ethanol, are known to be capable of destabilizing phospholipid membranes[24,31,32] and hence are considered to be good candidates. We are currently investigating this issue.

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