The Role of Ion-Phospholipid Interactions in Zwitterionic Phospholipid Bilayer Ion Permeation

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Methods

Materials

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) and 1,2-di-O-(9Z-octadecenyl)-sn-glycero-3-phosphocholine (diether-DOPC) were obtained from Avanti Polar Lipids Inc., Alabaster, USA. Sodium chloride, potassium chloride, ethanol and tris(hydroxymethyl)-aminomethane (Tris) were supplied by Sigma-Aldrich Australia. The electrodes and equipment to form the tethered phospholipid bilayers was obtained from SDx Tethered Membranes, Pty Ltd, Sydney, Australia.
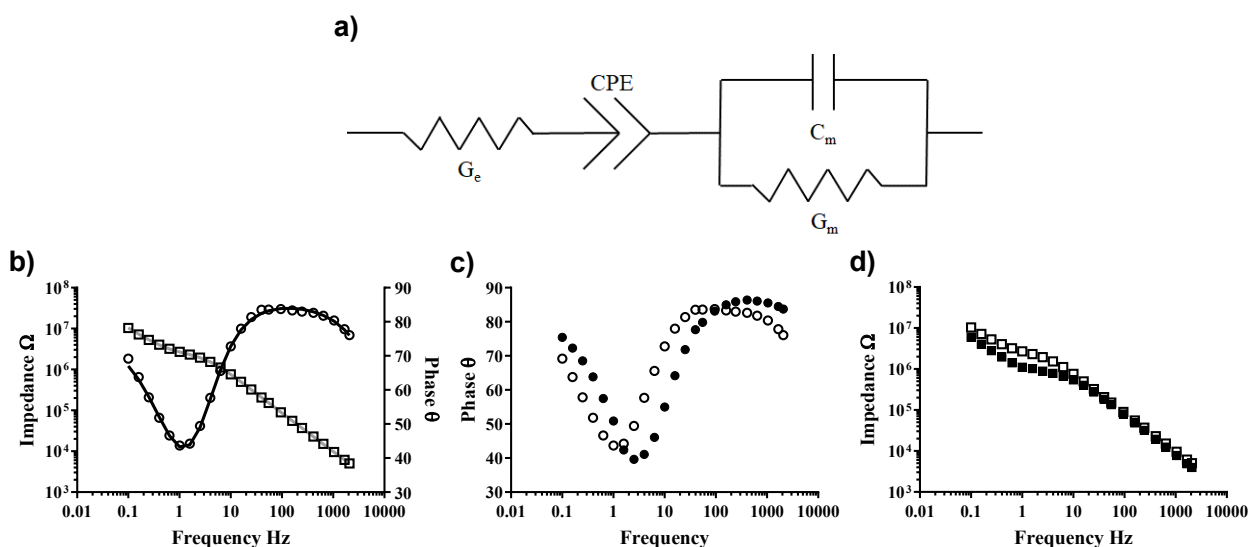
Methods tBLM/EIS

Tethered lipid bilayer membranes were formed following a standard procedure [1]. Briefly, tBLMs were made using pre-prepared monolayer coated gold slides. The monolayer coating chemistry comprised 10% benzyl disulphide eleven-oxygen- ethylene-glycol reservoir linkers with a C20 phytanyl group as tethers and 90% four-oxygen-ethylene-glycol reservoir linkers with a terminal OH group as spacers (catalogue reference T10™, SDx Tethered Membranes Pty Ltd, Sydney, Australia). A lipid bilayer was formed using a solvent-exchange technique [1] that employed 3 mM ethanolic solutions of either DOPC, SOPC, POPC or diether-DOPC lipids. All EIS experiments were carried out at room temperature, at which all four lipids are in the liquid crystalline state.

All experiments were performed with 10 mM Tris buffered solutions at pH 7 achieved by dropwise addition of 1 M HCl. All buffers were maintained at room temperature. To determine the effects of Na^+ and K^+ on membrane conductance (G_m), a series of titrations of the monovalent cation chloride salts were performed within the concentration range of 10 µM to 1 M. Following addition, each buffer solution was equilibrated on the membrane for ten minutes. Following each measure, the solution was exchanged by rinsing with a new solution of different ionic concentration through the 100 µm flow-cell cartridge supplied with the T10™ electrodes (SDx Tethered Membranes, Pty Ltd, Sydney, Australia).

The conductance (G_m) and capacitance (C_m) of the tBLMs were determined using a tethaPod™ swept frequency impedance spectrometer (SDx Tethered Membranes, Pty Ltd, Sydney, Australia) employing real-time modelling of the impedance profiles using tethaQuick™ software (SDx Tethered Membranes, Pty Ltd, Sydney, Australia). A resistor-

capacitor model was chosen to describe t G_m and C_m; with a constant phase element (CPE) in series to describe the reservoir region between the tethering gold surface and the membrane. A series resistor is included to model the electrolyte solution conductance (G_e). The equivalent circuit is shown in Fig. S1a and has been previously described[2]. A sequence of 50 mV peak to peak excitation periods were applied within the frequencies 0.1 Hz to 2 kHz using five steps per decade. G_m and C_m values were normalized to a 10 µM concentration of the relevant cation solution. For a detailed description of AC impedance spectroscopy methods used refer to Cranfield et al [2].

Figure S1. Equivalent circuit and examples of bode plots. a) The equivalent circuit used to model tBLMs where G_e is the conduction of the electrolyte solution, G_m the membrane conduction and C_m the membrane capacitance. CPE is the *constant phase element* representing the reservoir and gold tethering electrode. b) The measured impedance (□) and phase angle (○) for a DOPC tethered bilayer. The solid lines are fits calculated from the model and the dots are the experimental data. c) Phase minima shift from a 10 µM (○) to a to 1 M NaCl (●) solution. d) Impedance shift from a 10 µM (□) to 1 M NaCl (■) solution.

Statistical Analysis

An unpaired t-test was used to determine the difference in membrane conduction between Na^{+} and K^{+} at 1 M for each lipid type. Statistical analysis was performed with GraphPad Prism 7 software.

Molecular Dynamics simulations

All simulations were carried out using the MD simulation package GROMACS version 5.1.4 [3]. POPC and ions were described using the parameters of the ECC-POPC[4] and ECC-ion[5]

models, respectively. Water was described by the SPC/E model[6]. All simulations were subject to period boundary conditions. Simulations were carried out at 313 K and temperatures of the lipids and the solvent were coupled separately using a Nose-Hoover thermostat [7-8]. Semi-isotropic pressure coupling to 1 atm was achieved using a Parrinello-Rahman barostat [9] with a time constant of 2 ps. A time step of 2 fs was used and equations of motions were integrated with the Verlet algorithm [10]. The PME algorithm [11-12] was used to calculate long-range electrostatic interactions and a real space cut-off of 1.0 nm with grid spacing 0.12 nm was used. Lennard-Jones potentials were cut-off at 1 nm and a dispersion correction was applied to energy and pressure. The neighbour list was updated every 20 steps.
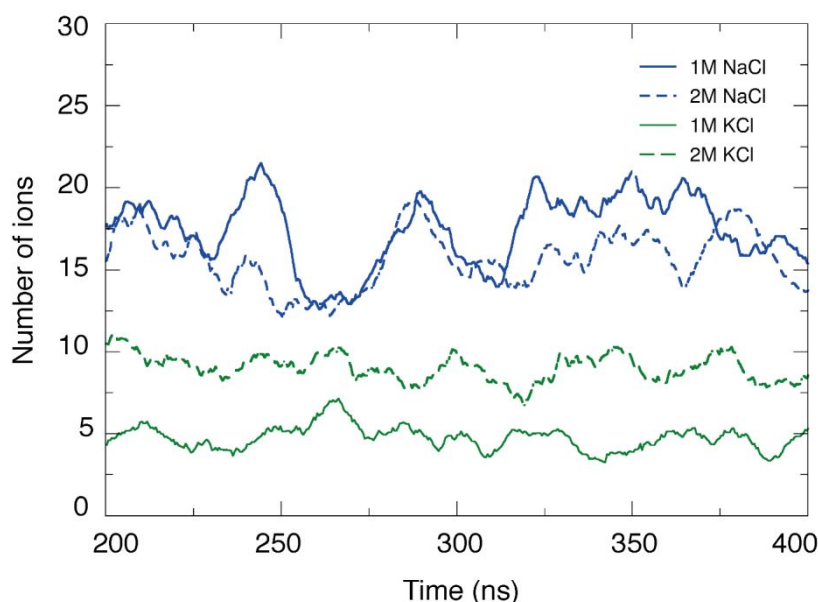
For simulations of a single POPC bilayer without ions, 1 M NaCl or 1 M KCl, simulations were started using the datasets deposited online as part of the NMRlipids project (nmrlipids.blogspot.fi) [13]. The systems contained 128 POPC lipids solvated with water only or water and 1 M NaCl or 1 M KCl. The existing 200-ns simulations were extended by another 400 ns. For simulations with 2 M NaCl or 2 M KCl, the last frame from the 1 M simulation was used and the necessary number of water molecules were replaced by the appropriate ions to reach the final 2 M NaCl or 2 M KCl concentrations. The system was energy minimised using a steepest descent algorithm. Both the 2 M NaCl or 2 M KCl system were simulated for 400 ns.

For the simulations of stacked POPC bilayers, two equilibrated bilayers obtained from the single POPC bilayers simulations were positioned in a box such that the there was a gap of at least 35 nm between the bilayers. The system was solvated with SPC/E water and the required number ions were added to the outer water layers to reach a concentration of 0.5 M NaCl or 0.5 M KCl. No ions were added to the water layer between the bilayer to create an infinite concentration gradient (see Fig 4 in the main manuscript). The system was minimised using a steepest descent algorithm. For both the 0.5 M NaCl and 0.5 M KCl systems, 20 independent simulations of 100 ns each were run using a random seed to assign the initial velocities.
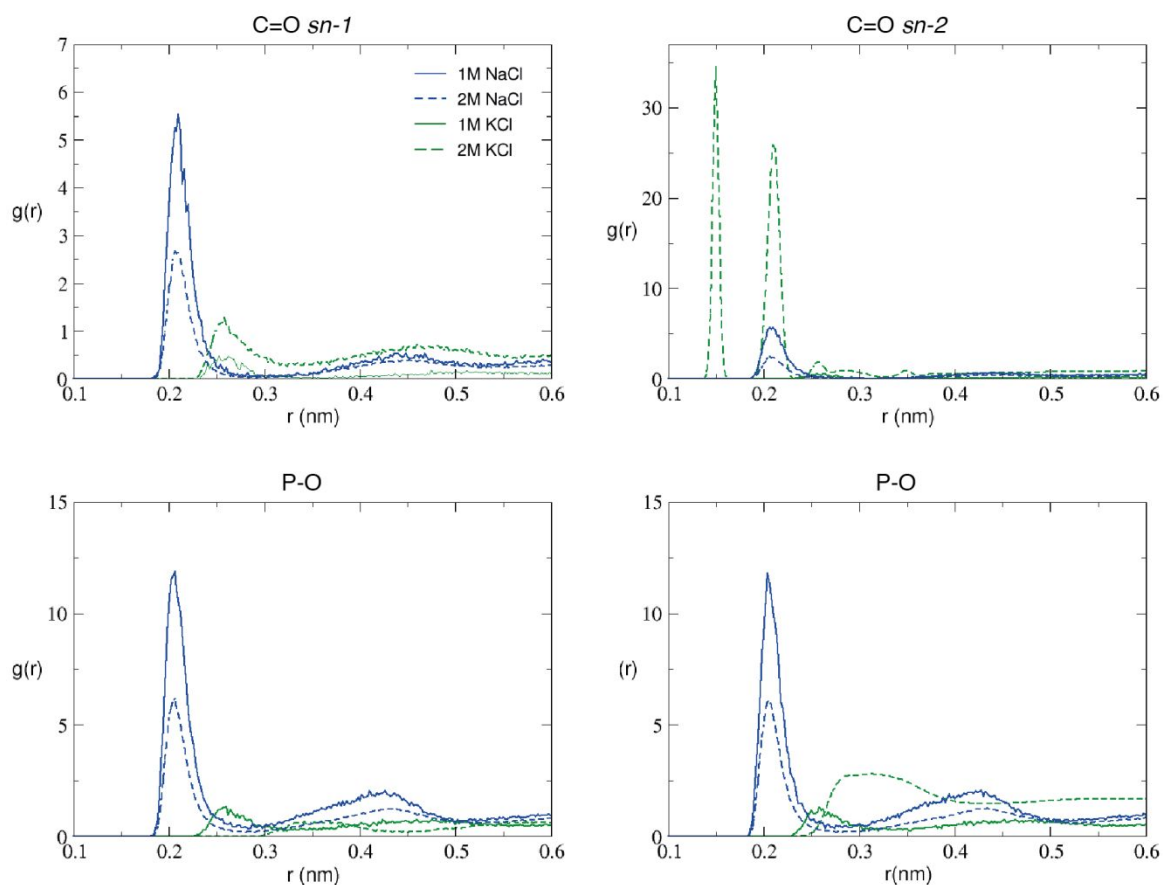
Analyses were carried out using GROMACS tools and python scripts with the MDAnalysis package[14]. Trajectories were visualised using VMD[15]. For the simulations of single POPC bilayer, unless otherwise stated, properties were calculated as averages over 1000 frames from the last 200 ns of the simulation. The area per lipid was calculated using the lateral

dimensions of the box (in the *xy* plane) divided by the number of lipids per leaflet. The bilayer thickness was derived from the electron density profiles of the phosphorus atoms of the system along the bilayer normal (taken as the *z* axis) and corresponded to the distance between the two maxima of the electron density. Surface bound ions were defined as ions that are within 0.35 nm of any heavy atom in the lipid head group or glycerol backbone. For analysis of ion-lipid or ion-lipid-water complexes, an ion was considered coordinated by a lipid oxygen atom or a water oxygen atom if the oxygen-ion distance was < 0.35 nm. Average distances between ions and oxygen atoms in the POPC molecules or water were obtained from the peak of the corresponding radial distribution functions.

For simulations of stacked bilayers, the entire 100 ns trajectory was used for analysis. The presence of a pores was defined as more than 50 water molecules in the hydrophobic section of the bilayer (defined by the average *z* position of the 3rd carbon atoms in the fatty acid chains). Pore size was estimated by averaging the number of water molecules in the pore over all frames where the pore was present. The duration of the pore was calculated from the first and last frame where the pore was present. The number of ions passing through the pores were calculated from counting the number of ions present in the middle water section between the bilayers at the end of the 100-ns simulation.

Figure S2. Ions at the water/lipid interface of a POPC membrane. Graphs show the number of Na+ and K+ ions within 0.35 nm of any heavy atom in the POPC lipid headgroup as a function of simulation time. Data was calculated using 500 frames from the last 100 ns of a 400-ns simulation of POPC in the presence of 1 M or 2 M NaCl / KCl. Average values are as follows. 1 M NaCl: 15 ± 3. 2 M NaCl: 17 ± 4. 1 M KCl: 5 ± 2. 2M KCl: 9 ± 4.

Figure S3. Radial Distribution Function for the Na⁺ and K⁺ binding to the carbonyl and phosphate oxygen in POPC. Data was calculated using 1000 frames from the last 200 ns of a 400-ns simulation of POPC in the presence of 1 M or 2 M NaCl / KCl. For the position of these oxygen in the POPC lipid see Figure 1 in the main article.

Table S1. Frequency and duration of water-filled pores in stacked POPC bilayers in the presence of 0.5 M NaCl or KCl. For each simulation system, 20 independent, 100-ns simulations were carried out. Pores were considered open if more than 50 water molecules were found in the hydrophobic core of a bilayer. The number of ions passing was determined by counting the ions in the water layer between the bilayers after the pore was closed.

Na+ (0.5 M NaCl)				K+ (0.5 M NaCl)			
Run	Duration of pore opening (ns)		Ions passing	Run	Duration of pore opening (ns)		Ions passing
	Upper bilayer	Lower bilayer			Upper bilayer	Lower bilayer	
1	-	-	-	1	-	8.8	5
2	14.4	-	5	2	33	26	5
3	11.4	-	5	3	36.2	-	6
4	6.6	-	5	4	13.4	-	5
5	24.2	-	4	5	15.8	-	6
6	-	-	-	6	-	3.6	6
7	5.6	-	5	7	-	3.4	6
8	13.8	-	7	8	8.2	-	5
9	-	15.6	7	9	15.4	-	6
10	-	14.8	6	10	-	-	-
11	11.2	-	4	11	-	31.2	7
12	-	14.6	7	12	-	12.8	6
13	10.4	-	4	13	11.6	-	4
14	-	-	-	14	17.4	-	5
15	-	14.4	5	15	16.4	-	7
16	-	25.6	6	16	-	10.6	4
17	21.8	-	7	17	17.2	-	7
18	-	12.4	5	18	-	-	-
19	5.0	-	6	19	24.2	-	6
20	10.4	-	5	20	8.8	-	5
Total pores	17/40 (42.5%)			Total count	19/40 (48%)		
Average duration	14.2 ± 4.8 ns			Average duration	16.5 ± 10.3 ns		
Average ions passing	5.4 ± 1.1			Average ions passing	5.6 ± 0.8		

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