Structure and Electroporation of Lipid Bilayers: a Molecular Dynamics Study

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Abstract

Pore formation in lipid bilayers subjected to a transverse electric field is studied by means of Molecular Dynamics simulations of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DOPC). The physical characteristics of the lipid membrane are crucial to understand the electroporation conditions. For example, addition of cholesterol (Chol) causes a substantial increment of membrane cohesion that results in an increase of the minimum electric field needed for membrane permeabilization. Instead, dimethyl sulfoxide (DMSO) is known to produce an opposite effect on membrane properties by increasing its fluidity and disorder that may open the possibility to facilitate the membrane electroporation process.

1. Introduction

Reversible pores in cell membranes can be induced by the application of an external electric field. This technique is referred as electroporemeabilization or electroporation [1], and has been used in numerous applications in biotechnology and medicine. For example, it is a common technique for introducing genetic material in cells and for delivering drugs across cells. The latter has resulted in a new tool to battle cancer, the electrochemotherapy, based on the application of pulses of ∼100 ms at a field strength of ∼1000 V/cm that induce the electroformation of reversible pores in the cell membrane, allowing the delivery of non-permeant drugs inside tumor cells [2,3].

Molecular Dynamics (MD) has been recently used in several works for elucidating the molecular mechanisms leading to pore formation in single-component membranes when an electric field is applied [4-6]. However, the understanding of electroporation in mammalian cell membranes requires the study of lipid bilayers containing cholesterol (Chol). Cholesterol is the most common lipid component in animal cell membranes and its role is fundamental for explaining their structural properties. It increases the order of fluid-phase phospholipid acyl chains and determines permeability, fluidity and mechanical properties of membranes. In this context, we have performed the molecular study of electroporation in bilayers containing different amounts of cholesterol, and the particular role of Chol in this phenomenon is analyzed. We report how Chol affects the main physical properties of a lipid membrane, mainly by increasing its lateral cohesion, and how this effect hinders membrane electroporation. This can be quantified by means of the minimum electroporation field, $E_0$ (the minimum value of the applied electric field that is required to induce pore formation), which is shown to increase with membrane cholesterol fraction [7].

Addition of membrane-fluidizing compounds could be conceived to counteract the effect of cholesterol on the electroporation phenomenon. For example, dimethyl sulfoxide (DMSO) is a small amphiphilic molecule that is known to fluidize and increase the permeability of cell membranes [8]. Here we have studied the effect of DMSO on cholesterol-containing bilayers and we have observed how the condensing effect of cholesterol is strongly attenuated by the addition of DMSO to the membrane solvent. Upon addition of DMSO, membrane area is expanded, lipid chains become disordered, and lipid organization is reduced. All these finding indicate that the electroporation process could be facilitated and that the minimum electric field required to electroporate the membrane could be strongly reduced in the presence of DMSO. The experimental validation of this conjecture could be of relevant application to the electrochemotherapy technique.

2. Protocols and Simulation Details

We carried out atomic-scale MD simulations for DOPC hydrated membranes mixed with 0, 20 and 40 mol % of Chol. All lipid bilayers were composed of a total of 128 DOPC molecules together with the corresponding amount of...
Chol molecules, homogeneously distributed in the two leaflets. Each bilayer system was also run in the presence of 1 mol% and 5 mol% of DMSO in the solvent. In all cases, the number of solvent molecules was fixed to 6186, but the molar fraction of DMSO in the water/DMSO mixture is varied.

The simulations were performed using the GROMACS v.3.3.1 software package [9], and they were carried out in the NpT ensemble at p=1 atm and T=310 K. Temperature and pressure were controlled by using the weak coupling method. The pressure coupling was applied separately in the bilayer plane (xy) and the perpendicular direction (z). The SETTLE algorithm was used to preserve the bond lengths in water molecules, whereas the lipid bond lengths were constrained with the LINCS algorithm. The united-atom force field parameters used in our simulations have been extensively tested and verified (see Ref. [7]). A cutoff distance of 1 nm was used for the Lennard-Jones interactions whereas electrostatic interactions were handled using the particle-mesh Ewald method with a cut-off of 1 nm. Periodic boundary conditions were used in all three directions, and the time step was set to 2 fs. All simulated membranes are in a fluid state. Each membrane is first equilibrated after 10-40 ns depending on the system [7], and production runs of 25-50 ns have been performed. Equilibration of the bilayers was determined by monitoring the membrane area and different mass density profiles.

3. Results

The molecular mechanism of membrane electroporation has been elucidated by means of numerical MD simulations of simple lipid bilayers subjected to a transverse electric field. According to these studies, the disordering of the water molecules at the membrane interface due to the applied external field may eventually generate a water defect that initiates pore formation. Such water defect can develop into a water column penetrating into the inner region of the bilayer (hydrophobic pore). Further on the lipid head groups reorient in order to cover the initial water column and form a hydrophilic pore (see the sequence in Figure 1). The kinetics of these stages has been characterized as well as the pore collapse when the applied field is removed [6].

The influence of the electric field on the water molecules close to the membrane interface is the main factor to promote the initiation of a pore. Therefore, the probability of pore formation increases at larger fields. However, intuition predicts that other factors, as for example the membrane compactness, may also have an influence on the electroporation process. For example, it is expected that for a given applied field, the more condensed membranes, the smaller probability of being porated. Two examples of this prediction are inspected in the following subsections.

Figure 1. Sequence of a pore dynamics in a DOPC/40%Chol bilayer subjected to an electric field E=750 mV/nm. Water molecules are plotted with red and white sticks, DOPC phosphate and Chol hydroxyl groups with orange and purple beads, respectively. DOPC tails and Chol molecules are plotted using blue and purple sticks, respectively. In lateral views only one DOPC and Chol molecules are shown while in top view water molecules are not included.

3.1 Effect of Cholesterol on Membrane Electroporation

Addition of cholesterol to lipid membranes is known to alter their structural properties, mainly by condensing them. Such condensing effect can be captured in our simulations [7]. For example, Chol causes the area per lipid, A_{PC}. 

to decrease with cholesterol fraction (Table 1). As expected, a decrease in area per lipid is accompanied by an increase of the bilayer thickness (see P-P distances in Table 1). The molecular mechanism that leads to membrane condensation is related to the ordering ability of cholesterol. The value of the averaged deuterium order parameter, $<-S_{CD}>$, indicates the lipid acyl chain ordering. The values for the averaged tail order $<-S_{CD}>$ reported in Table 1 reveal, as expected, an increasing chain ordering with the cholesterol contents. The study of the transverse structure of the membrane system was addressed by computing the mass density for different molecules and lipid groups as a function of the 'z'-axis. Figure 2 shows the typical features obtained from diffraction experiments, namely, two pronounced peaks in the phosphate group positions and a minimum in the middle of the bilayer. Moreover, when cholesterol is present, the transversal ordering is increased (more pronounced peaks) and the lipid density is reduced in the region where cholesterol resides, i.e., cholesterol pushes the lipids aside from the bilayer center.

In order to compute the minimum electroporation field, $E_0$, each membrane simulation was run independently for 25 ns in triplicate, and a transverse electric field was fixed. In the case that none of the three replicas of the system displayed pore formation after 25 ns, the field was increased in 25 mV/nm and so on. When at least one of the three replicas was porated, the value of the applied field was taken as $E_0$. A clear correlation is found between the behavior of the structural properties discussed above and the dependence of $E_0$ with cholesterol fraction: $E_0=325$, 475 and 750 mV/nm for 0, 20 and 40 mol% Chol, respectively (see Ref. [7] for more details). Membranes with smaller area per lipid and more ordered and packed acyl chains require stronger electric fields in order to be electroporated. Cholesterol condensing effect acts, therefore, against electropore formation.

### 3.2 Effect of DMSO on Membrane Properties

Our simulations with DMSO show the main effects of this component on Chol-containing bilayers. The analysis of the mass density profiles plotted in Figure 2 reveals that DMSO is preferentially located in the inner membrane/water interface region, close to the phosphate groups as it corresponds to its amphiphilic nature. Figure 2 also depicts that the incorporation of DMSO to the membrane breaks the transversal membrane ordering: the lipid density profiles are smoothed, particularly at low Chol fractions. It has to be also noticed that for a given Chol fraction, the addition of DMSO increases water penetration into the membrane (Figure 2).

The equilibrium values for the main structural properties of our simulated membranes with 1 mol% and 5 mol% DMSO are provided in Table 1. The main effect of DMSO is to increase the total membrane area, $A$, counteracting the condensing effect of cholesterol (Table 1). Notice that the values for the area per lipid, $A_{PC}$, are not computed for DMSO-containing systems since insertion of DMSO into the bilayer does not allow a correct assignment of the area corresponding to each membrane component. DMSO also disorders the hydrophobic region of the membrane as it can be observed by looking at the averaged deuterium parameter $<-S_{CD}>$ (Table 1). In turn, the latter causes a decrease of the bilayer thickness (see P-P distance in Table 1). Finally, it has to be noticed that cholesterol lessens all mentioned effects: larger DMSO fractions have to be considered to obtain similar effects than in cholesterol-free membranes. For example, addition of 5 mol% of DMSO in the solvent phase causes an increase of about 30% in area and a reduction of 13% in thickness for Chol-free and 20 mol% Chol membranes. Instead, the variations are smaller for 40 mol% Chol bilayers; we obtain a 12% of area increase and a 3% of thickness reduction.

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<th>%mol Chol</th>
<th>%mol DMSO</th>
<th>A (nm$^2$)</th>
<th>$A_{PC}$ (nm$^2$)</th>
<th>P-P dist (nm)</th>
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According to the obtained results, the influence of DMSO on the structure of lipid bilayers counteracts the condensing effect of cholesterol, and this indicates that addition of DMSO to the membrane environment may facilitate...
electroporation. In order to confirm this prediction, the numerical computation of the values for the minimum electroporating field \( E_0 \) in DMSO-containing bilayers is in progress.

Figure 2. Mass density profiles in a DOPC bilayer with (a) 0 mol\%, (b) 20 mol\% and (c) 40 mol\% Chol fraction. Thin and thick lines correspond to 0 mol\% and 5 mol\% DMSO systems, respectively. The profiles are represented in a scaled distance respect to the bilayer center where the maxima of phosphate groups are fixed at \( Z'=\pm 1 \). The profiles correspond to water (black), DOPC (red), Chol (green) and DMSO (blue).

4. Conclusions

The physical properties of a lipid membrane determine its propensity of being porated by a transverse electric field, and this can be quantified by the evaluation of the membrane minimum electroporating field \( E_0 \). By means of MD simulations we have shown that Chol increases membrane cohesion, and as a result, a large value for \( E_0 \) is obtained when adding Chol to the membrane. Instead, we have reported that DMSO fluidizes and disorganizes the lipid membrane, so the electroporation process could be facilitated and the value of \( E_0 \) is expected to decrease upon addition of DMSO to the membrane environment. Experimental validation of this conjecture is in progress and could be of relevance to improve electrochemotherapy technique.

6. References