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Modeling the induction of lipid membrane electropermeabilization

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Abstract

Experiments show significant effects of an electric field on lipid membrane, leading to a pore formation when a high intensity field is applied. The phenomenon of electroporation is preceded by the induction and expansion of defects, responsible for the pre-pore excitation. We examine the mechanism of the induction of the field-driven defects by Monte Carlo simulations. The study is based on the improved Pink's model, which includes explicit interactions between the polar heads and energy of interactions between the heads and the field. No anomalous deformation of the molecules is considered. The study, provided for bilayer dipalmitoyl-phosphatidylcholine (DPPC) membrane in the gel (300 K) and fluid (330 K) phases, shows dependence of the membrane conformational and energetical state on the value of the electric field. We observe that the electric field affects the number of molecules in the gel and in the fluid states. In the layer at the negative potential, when the transmembrane voltage is above $U_c \approx 280$ mV, lipid heads abruptly reorient and the number of local spots with fluid conformation increases. The other layer slightly tends to tighten its structure, producing additional mechanical stress between layers. Lipids showed complete insensitivity to the electric field within physiological limits, $U < 70$ mV.

Keywords: Model membrane; Electric field; Monte Carlo; Electroporation; Pre-pore

1. Introduction

A high intensity electric field applied to a lipid membrane changes its properties, leading to the creation of conducting hydrophilic nanopores. This phenomenon, called electroporation, is preceded by pre-pore membrane excitation and creation of hydrophobic pores. In the process, the hydrophobic pores can eventually make the transition to hydrophilic pores [1–8].

Pre-pore excitation, which is of our special interest in this paper, develops either as a first stage of the full electroporation process or it accompanies the application of electric impulses which are too short for the pores to appear. At this stage the membrane evolves into an anomalous excited state (a "stress state") observed for tens of minutes. Although no pores appear, the membrane displays large conductance and current fluctuations, even at low voltage [2]. The first step of the pre-pore excitation, called the "induction step" [3], probably results from local defects which appear when the field reaches a critical value of about 200 mV, the value depending also on the buffer composition. A molecular mechanism leading to these defects is still not clear.

The most commonly accepted explanations of this phenomenon are based on variations of the electrocompression theory. Due to this theory, a high field causes continuous deformation of lipid molecules, which assume anomalous conformations different from those occurring in typical gel or fluid phases [9]. As a result an overall decrease in membrane thickness appears. However, the electrocompression theory is [2] still debatable. Although for smaller transmembrane potential V the capacitance increases by 3–5%, it may be due to the different membrane geometry, rather than altered dielectric properties of the membrane [2] (and references therein). This observation supports the idea that the electric field may enlarge distances between molecules, with no large-scale compression of the hydrophobic part. There is a possibility that local defects, giving rise to pre-pore excitation, are not resulting from electrocompression. This idea is

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explored in this paper by Monte Carlo simulations studying the field-driven effects.

In our model, we assume that lipid chains accept only typical conformations represented by 10 energetical classes [10,11]. At temperature $T = 300$ K the conformation characteristic for the gel phase (all-trans) prevail, at $T = 330$ K fluid conformations are in the majority. The electric field affects the membrane only through interactions with polar heads, represented by mobile dipoles. Since conformation of lipid chains is sensitive to the polar part of the molecule, the electric field may have an influence on the hydrophobic part of the membrane, too. The total energy of the membrane is calculated in accordance with Pink's model [10], which involves interactions between the hydrocarbon chains and internal conformational energy. However, in place of the surface energy term, explicit electrostatic interactions for polar parts were added [12–18]. Hence, interactions between molecules and electric field could also be considered. The model includes the energy of interactions with the electric field as well. Monte Carlo (MC) simulations allow to choose the energetically favorable state, representative for each value of external electric field. The model can be used to observe an influence of the electric field on the membrane structure. The model is sensitive to the ionic strength of the buffer [19,20], whose effect is observable in electropermeabilization [3].

2. Method

The bilayer was modeled as two triangular lattices. The direction of the electric field is perpendicular to the membrane surface (Fig. 1). The layer at the positive potential (PL) and the layer at the negative potential (NL) were treated and calculated separately. No interactions between layers or molecule migration (flip-flop) were considered. Since all the nodes were occupied, the model did not allow to study a possibility of an electropore appearance.

Each node of the lattices represented an acyl chain and two chains were attached to one lipid head group. The whole molecule could rotate 180° around the normal to the membrane surface. The chains were treated separately and each of them could take one of ten possible states based on its conformational energy. The number of actual conformations in each state α was represented by degeneracy D_α ranging from $D_1 = 1$ for all-trans conformation to $D_{10} = 354{,}294$ for the fluid state [11]. Chain conformation is defined by the angles between C–C bonds. The angle was approximated as 35° or 145° to the bilayer normal for trans bonds and 90° for gauche bonds. The chain closer to the polar head is effectively shorter of two C–C bonds (two bonds in the *β*-chain are directed along the membrane surface) [21], which was incorporated into the model. The distance between positive charge at N-atom of the choline group and negative at the P-atom was fixed, $d_{\mathrm{PN}} = 5 \cdot 10^{-10}$ m. Each chain from the lattice interacted with six nearest neighbors.

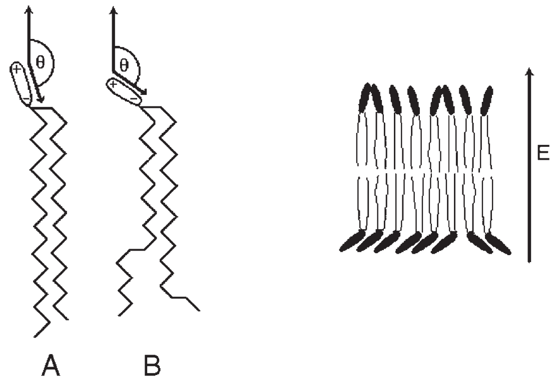

Fig. 1. Exemplary conformations of lipids in the electric field with an angle θ between a field direction and a head (Eq. (9)). A. Both chains in all-trans conformation, the angle of C–C bond to the membrane normal is 35° or 145°, standing head (78°). B. Both chains have one gauche C–C bond with the angle 90° to the bilayer normal, lying head (30°).

In the original Pink's model, the structure of lipid polar heads was not considered and the surface energy term represented the dipolar interactions responsible for the membrane integrity. This approach provided good models of the gel–fluid transition but it did not allow the study of the influence of ions on lipid membrane. The experiment presented here is based on the model in which the dipolar structure of the head was explicitly incorporated so that the polar part of the molecule could contribute to the final state of the membrane. In the model lipid heads were zwitterionic, represented as dipoles. They could assume one of two possible tilts toward the membrane surface 78° (standing) and 30° (lying), Fig. 1. This assumption modeled two extreme positions of the heads [16] (and references therein). Polar heads could rotate toward their nearest six neighbors (nodes). Electrostatic interactions between dipoles, incorporated into the model, included 14 neighboring dipoles [17]. Periodic conditions were imposed on the boundaries of the whole lattice.

The Hamiltonian of the studied system involves 4 terms — the energy of van der Waals interactions H_{vdW}, the conformational energy H_{conf}, the energy of electrostatic interactions between polar heads H_{dip}, and the energy of interactions with electric field H_{e}.

$$H = H_{\mathrm{vcdW}} + H_{\mathrm{conf}} + H_{\mathrm{dip}} + H_{\mathrm{e}} \tag{1}$$

$$H_{\mathrm{vdW}} = -\frac{J_0^M}{2} \sum_{i,j=1}^{N} \sum_{n,m=1}^{10} f(r_{nm}) S_n S_m L_{ni} L_{mj} \tag{2}$$

J_0^M (denotes the interaction energy between two parallel chains in all-trans conformation. Lattice coordinates are i (site index ranging from 1 to N) and j (index of 6 sites neighboring with site i). The index of chain conformational state n and chain conformational state m range from 1 (all-trans) to 10 (fluid) [10]. The distance r_{ij} between the two chains at sites i and j depends on their conformational states. State operator L_{ni} of the chain located in site i equals 1 if the i-th chain assumes conformation n, and 0 otherwise.

Order parameter S_n for the acyl chain in conformation n yields

$$S_n = \frac{\sum_p S_{np}}{\sum_p S_{1p}} \tag{3}$$

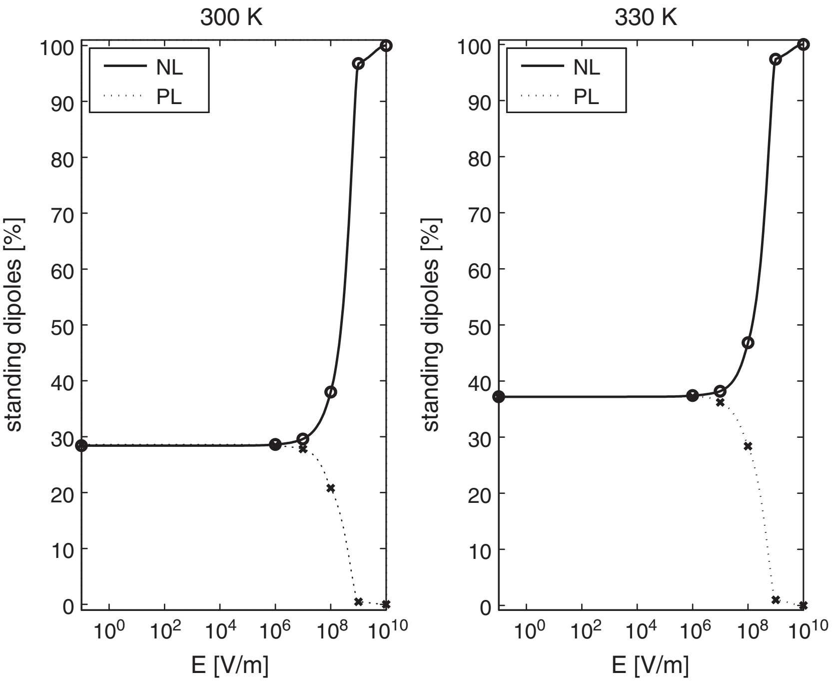

Fig. 2. The rate of the heads in standing configuration shows two limits — negligible field influence if $E<E_0\approx 10^7$ V/m and rapid reorientation of heads if $E>E_c\approx 0.5\cdot 10^8$ V/m. Negative layer (solid) and positive layer (dotted) in A. $T=300$ K and B. $T=330$ K.

where S_{np} order parameter of the p-th C–C bond

$$S_{np} = \frac{1}{2}(3\cos^2\theta_{np}-1) \tag{4}$$

p is the index of C–C bond in the chain. The bond is characterized by the angle θ_{np} between the bilayer normal and the normal to the plane spanned by the p-th CH_2-group of the chain.

Distance dependence of the van der Waals interactions between the chains in the m-th and n-th conformations r_{nm} is expressed by $f(r_{nm})$

$$f(r_{nm}) = w_n\left(\frac{r_1^2}{r_n r_m}\right)^{5/2} \tag{5}$$

where r_n denotes the radius of the space occupied by an average chain in the n-th conformation, w_n is a weakening factor, $w_{10}=0.4$ for chains in the fluid state and $w_n=1$ if $n\neq 10$. The factor was introduced by Mouritsen [11] in the modification of Pink's model to provide good agreement between the model and experimental data.

The conformational energy H_{conf} is defined according to the internal energies E_n of the predefined [11] chain conformations

$$H_{\text{conf}} = \sum_{i=1}^{N}\sum_{n=1}^{10} E_n L_{ni} \tag{6}$$

The interactions between polar heads H_{dip}, which are due to electrostatic interactions, are given by:

$$H_{\text{dip}} = \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{14}\sum_{\alpha,\beta=1,-1}\frac{\alpha Q_{\alpha i}\beta Q_{\beta j}\exp(-\kappa r_{\alpha i\beta j})}{4\pi\varepsilon\varepsilon_0 r_{\alpha i\beta j}} \tag{7}$$

where ε is the electrolyte dielectric constant, ε_0 is the permitivity constant and $Q_{\alpha i}$ is the effective polar head charge with $Q_{\alpha i}=q/2$, where q is the actual dipole charge which equals an elementary charge [16] and α denotes the charge sign, $\alpha=1$ for a positive charge and $\alpha=-1$ for a negative. The distance between charges α and β of the dipoles at sites i and j is represented by $r_{\alpha i\beta j}$.

The inverse of Debye length κ defines the range of electrostatic interactions with screening

$$\kappa = \sqrt{\frac{2z^2F^2c}{\varepsilon_0\varepsilon RT}} \tag{8}$$

where $z=1$ is the valency of polar head charge, F is Faraday's constant, T is the absolute temperature, c is the ionic strength of the solution and R is the ideal gas constant.

The energy H_e reflects the interactions between the polar parts of the molecules and the electric field

$$H_e = \sum_{i=1}^{N} Q_i dE(1-\cos(\theta)\big) \tag{9}$$

where Q_i is a dipole charge, d is its length, θ denotes the angle between the directions of the electric field E and the dipole (Fig. 1). The variable E represents an effective electric field acting on lipid molecules, which involves all possible effects occurring during electropermeabilization (e.g. interfacial phenomena, divider effect, etc.) incorporated into one variable. Therefore, it may differ from the value of the external field set by an experimenter.

The influence of the electric field on the bilayer lipid membrane was analyzed in the range $0-10^{10}$ V/m. The membrane was examined at temperatures characteristic for gel and fluid phases, $T=300$ K and $T=330$ K, respectively. The conformation of lipid molecules was studied based on the tilt of polar heads, number of chains in two extreme energetical states — the most condensed gel (all-trans) and relaxed fluid conformations, and the average area occupied by one lipid molecule. We investigated if the field causes noticeable changes in the membrane structure that may lead to electropermeabilization in the pre-pore stage of electroporation. As assumed in the model, the electric field affects the membrane through interactions with polar heads. The heads, represented by mobile dipoles, are expected to reorient in the non-zero electric field. Positively charged choline groups are attracted by the field of lower potential so the heads from NL tend to assume a standing configuration (78°). For the positive field, in contrast, choline groups are repelled by the field. Therefore, the heads from PL should have a tendency to assume a lying configuration (30°). Additional effect on the conformation may have the energy of interactions between the electric field and the membrane.

The simulations were carried out for a bilayer dipalmitoyl phosphatidylcholine (DPPC) membrane with 16 C atoms in each acyl chain, represented by a hexagonal lattice with 10×10 nodes and periodic conditions imposed on the boundaries of the lattice. A canonical ensemble was assumed. The system was equilibrated for 1000 Monte Carlo steps per site, then 10,000 steps per site were performed.

A series of microconfigurations, which is a Markov process, was selected by means of Metropolis method [11].

3. Results and discussion

The simulations confirmed that the tilt of the heads is sensitive to the external electric field (Fig. 2), consistent with the experiments on supported lipid membranes mimicking field-driven membrane processes in cells [24]. Interestingly, in our study the dipoles do not react to attracting and repelling forces if the field value is below $E=10^6$ V/m. Noticeable effects appear only above $E_0\approx10^7$ V/m. When the field is further increased, rapid reorientation of dipoles starts at the critical value $E_c\approx0.5\cdot10^8$ V/m and at $E=10^{10}$ V/m all heads assume the expected final orientations. In two studied temperatures, characteristic values of E do not depend on the temperature. The number of reoriented dipoles is greater for NL since more heads are in the lying position if no electric field is applied.

Similarly, the field effect on other parameters related to the membrane conformation, such as the average number of chains in gel (all-trans) conformation (Fig. 3), the average number of chains in fluid conformation (Fig. 4), and the average surface area per one chain (Fig. 5) is negligible if the applied field is lower than E_0. This finding suggests that it is not energetically favorable for the membrane to change its conformation when the electric field is not very high. The value of E_0 corresponds to the membrane potential $U_0\approx50$ mV, which indicates that at physiological values of membrane potential, conformation of the plasma membrane in cells is unaffected by the field. The value of E_c is also consistent with the electroporation experiments [2], which show that the membrane is endangered by electroporation if the membrane potential is above 250 mV [2].

An increase in the field intensity above E_c leads to a sudden transition of the membrane characteristics, generated by the displacement of the heads and additional energy of interactions between the field and polar parts of lipid molecules. The effects are different for each layer and depend on the temperature.

At temperature $T=300$ K, in NL, the average number of chains in all-trans conformation decreased from 41.4% to 40.6%

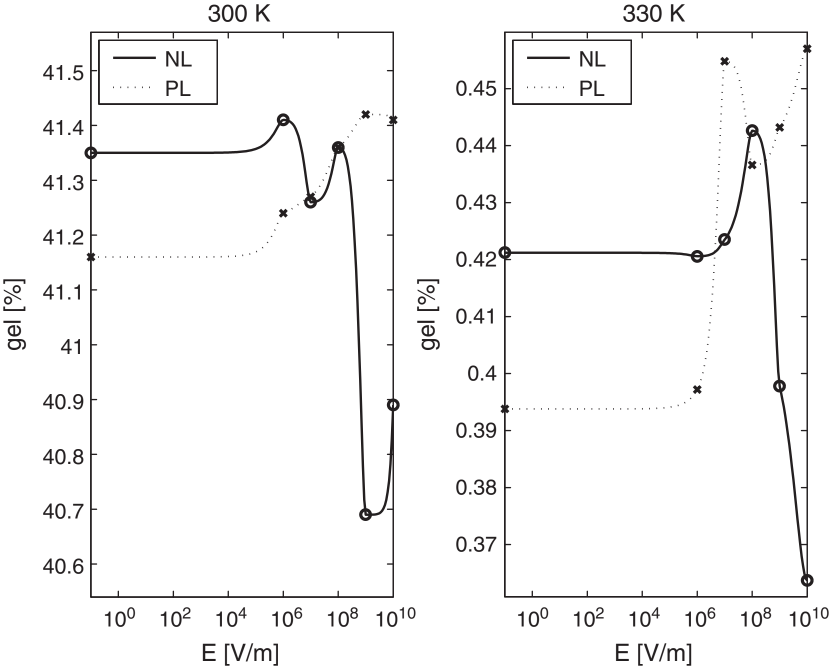

Fig. 3. The rate of the chains in gel (all-trans) conformation depends on the field value for $E>E_c\approx0.5\cdot10^8$. Negative layer (solid) and positive layer (dotted) in A. $T=300$ K and B. $T=330$ K.

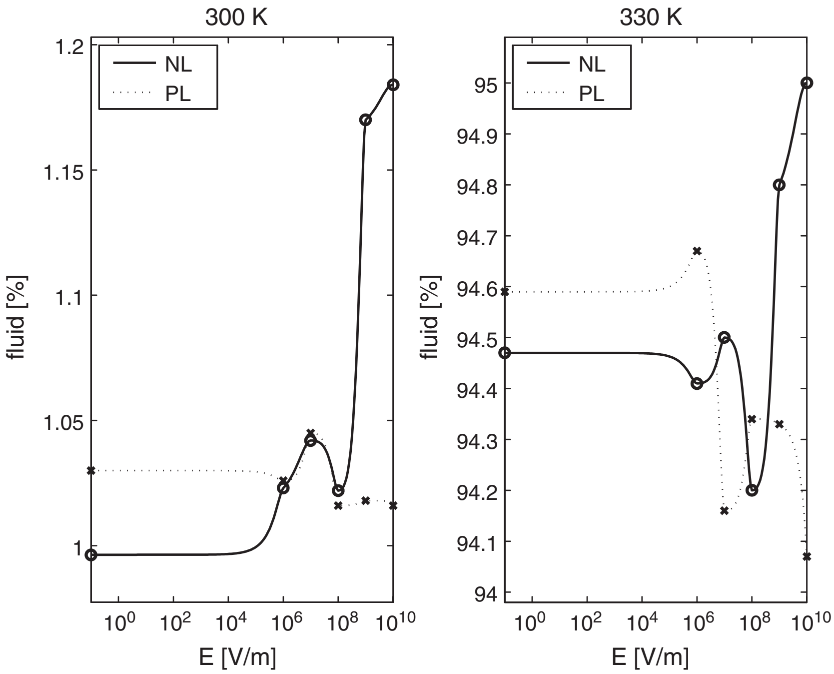

Fig. 4. The rate of the chains in fluid conformations. Negative layer (solid) and positive layer (dotted) in A. $T=300$ K and B. $T=330$ K.

(Fig. 3 left panel, solid) and the average number of chains in fluid conformation increased from 1% to 1.2% (Fig. 4 left panel, solid). Chains in conformations initially other than gel or fluid also tended to assume a less compact conformation, which finally resulted in increased average area occupied by a single chain (Fig. 5 left panel, solid). Total area taken by one lipid molecule increased on average by 0.1 nm^2. A different response came from the layer at positive potential. The average number of chains in all-trans conformation increased from 41.2% to 41.4% (Fig. 3 left panel, dotted). However the average number of chains in fluid conformation remained almost constant (Fig. 4 left panel). In total, the average area occupied by lipid molecule in PL (Fig. 5, left panel, solid) decreased by 0.02 nm^2.

At temperature $T=330$ K the tendency is similar although compression in PL is more pronounced than in lower temperature. It was observed that the average number of chains in all-trans conformation decreased from 0.42% to 0.36% (Fig. 3 right panel, solid), the average number of chains in fluid

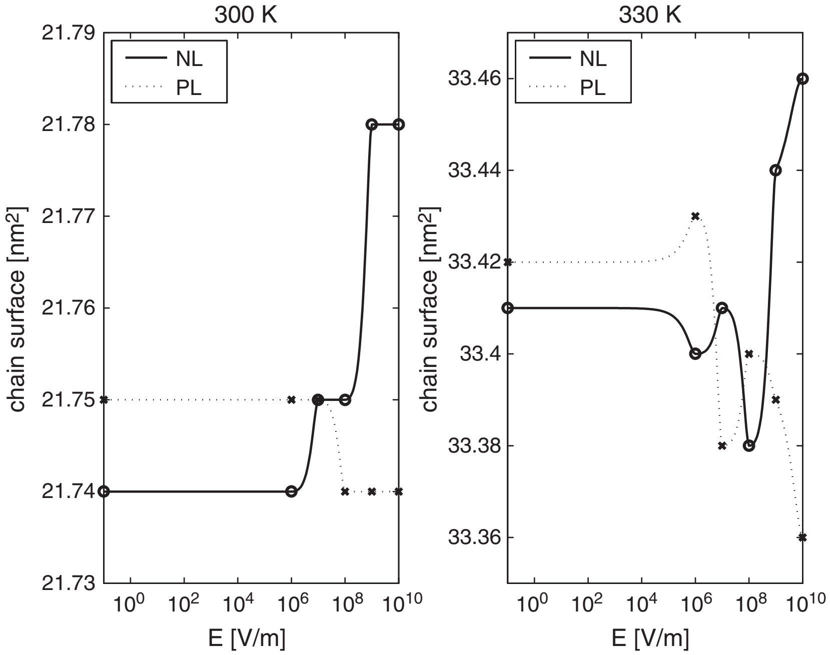

Fig. 5. The average area occupied by a single chain. Negative layer (solid) and positive layer (dotted) in A. $T=300$ K, expanded negative layer and unaffected positive layer; B. $T=330$ K, opposing tendencies in two layers.

conformation increased from 94.5% to 95% (Fig. 4 right panel, solid). The total area taken by one lipid molecule increased on average by 0.15 nm^2 (Fig. 5 right panel, solid). In PL, the average number of chains in all-trans conformation increased from 0.4% to 0.45% (Fig. 3 right panel, dotted). The average number of chains in fluid conformation decreased from 94.6% to 94% (Fig. 4 right panel, dotted). In total, the average area occupied by lipid molecule in PL decreased by 0.12 nm^2 (Fig. 5 right panel, dotted).

The simulations showed that in the gel temperature the structure of the layer at a negative potential gets less compact with increased distances between molecules, while the other layer remains almost unaffected by the field. Although the expanding effect is much less significant than in the phase transition, it is very distinct and reproducible, indicating that for energetical reasons a less compact structure of the negative layer is more likely. At higher temperature, $T = 330$ K, the expanding tendency for NL is comparable with the compression of PL. Consequences of the opposite processes in two layers could not be investigated within this model, which treats the layers separately. It is possible however that such an asymmetry produces an additional mechanical stress between layers, and it may result in additional curvature of the membrane or encourage displacement of molecules from PL to NL in the flip-flop process. Finally, it may contribute to the appearance of defects in the membrane leading to the creation of pores [22,23]. It could be noted that just below the critical field value E_c opposite effects appear, the tendencies of NL to expand and PL to compact are temporarily reversed for both, separately calculated, layers. This effect is more evident for the higher temperature.

4. Conclusions

The Monte Carlo simulations, based on modified Pink's model with explicitly incorporated interactions between polar parts and the energy of the electric field, examined the influence of the electric field on the structure of lipid bilayer. We investigated whether the field alters the membrane in the prepore stage of electroporation in a way that may account for the "induction step" of electropermeabilization.

The model did not consider any electrocompression effects, chains could assume only typical conformations occurring in the studied temperatures. The model assumed that the electric field affects the membrane through interactions with the polar heads, introducing the energy of interactions and changing the orientation of the polar heads. Since the conformation of the hydrophobic part of the membrane is sensitive to the state of the hydrophilic part, the chains should be responsive to the field, too.

The simulations confirmed that the tilt of the heads is sensitive to the external electric field as expected. However, the orientation of the dipoles changes only if the field exceeds the value $E_0 = 10^7$ V/m, which corresponds to the membrane potential $U_0 \approx 50$ mV. Similarly, the field effect on other parameters related to the membrane conformation, such as the average number of chains in the gel and fluid conformations and the average area occupied by a single lipid, is negligible if the applied field is lower than E_0. This finding suggests that it is energetically favorable for the membrane lipids to maintain the same conformation when the electric field is within physiological limits of the membrane potential occurring in cells. This observation is especially relevant for plasma membranes of excitable cells, indicating that lipids maintain the same characteristics throughout the action potential. When the field was increased over the critical value $E_c \approx 0.5 \cdot 10^8$ V/m (corresponding to $U_c \approx 250$ mV), the reorientation of the polar heads became very rapid. At this field value, more distinct changes of membrane conformation could be observed — increased number of spots with the molecules in fluid conformation and the asymmetry between layers, which may stimulate appearance of the defects facilitating subsequent electropermeabilization.

The critical values of electric field accompanying structural changes in the membrane are comparable with experimental data, showing the physiological limit of negligible field influence and the limit of abrupt reconfiguration. However, the significance of the observed structural changes is still debatable. It remains an open question whether the structural changes observed from the model may entirely account for the first stage of the electropermeabilization, or contribute to this phenomenon in concert with other effects.

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