MODELING THE EFFECT OF IONIC STRENGTH ON DURABILITY OF LIPID MEMBRANE IN THE GEL PHASE*

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Dedicated to Professor Andrzej Fuliński on the occasion of his 70th birthday

Experimental evidence shows significant effect of ionic concentration of aqueous buffers on the membrane stability, for example during electroporation experiments. Also, the lateral diffusion coefficient is sensitive to ionic strength. We study the effect of ionic strength on lipid membrane by modified Pink model, applying Monte Carlo simulations for a lattice of 50 lipid molecules. The study is provided for the model dipalmitoylphosphatidylcholine (DPPC) membrane in the gel phase at the fixed value of dielectric constant. The study in the range of 10–3000 mM, shows a decrease of repulsive interactions between polar heads, accompanied with an increase of attracting van der Waals interactions between acyl chains. Additionally, the chains assume more stable conformation with lower conformational energy. Simulations show rising number of standing polar heads, which may indicate better accessibility of ions from the solution to the polar part of the molecules and their consequent binding.

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1. Introduction

Properties of biological membranes or embedded ion channels are often studied by means of model lipid membranes surrounded by aqueous buffers. The buffers contain numerous monovalent or divalent ions in various concentrations. It has been observed that the concentration alone changes properties of the membrane, the higher ionic strength the more durable membrane. For example, membranes located in high electric field, which undergo electroporation [1–3], are more stable in high ionic strength [4, 5]. This effect provided motivation for this study. The phenomenon of electroporation is attracting interest because of its application to biotechnology and medicine. It is currently used as a simple and relatively nontoxic method for introducing exogenous macromolecules (such as DNA, RNA, proteins, drugs, and fluorescent probes) into cells of various types [6]. Electroporation has also been observed as an effect of large defibrillation chocks applied to the cardiac tissue [7, 8]. Another consequence of increased ionic strength is experimentally observed decrease of the membrane lateral diffusion constant [9] and changes of the membrane physical properties [10].

However, the exact mechanism of these phenomena are not fully understood. The interactions between ions and uncharged lipids are considered as weak especially for monovalent ions. The dissociation constant for Na^+ is in physiological limits (100–500 mM) [11]. Then, little is known about interactions between ions from buffers with uncharged or zwitterionic lipids, as well as influence of their concentration on interactions between lipid molecules in the membrane. Molecular dynamics simulations [9] suggest a tight binding of sodium ions to the carbonyl oxygens producing complexes of greater size and reduced mobility, increased deuterium order parameter of the fatty acyl chains, and creation of a diffusive capacitor responsible for a strong electric field near the lipid headgroups.

Monte Carlo (MC) simulations of the molecular interactions between lipid molecules for different ionic concentrations can provide another perspective of this phenomenon. Molecular interactions alone strongly influence dynamical and structural characteristics of lipid membranes, its stability and susceptibility to disintegrating factors. Studies of energetic profile of lipid membrane, its structure and phase transitions can be obtained by Monte Carlo simulations based on Pink model [12–22]. In these models lipid membrane is treated as a hexagonal lattice where each node represents an acyl chain. The chains are considered as separate and each of them can assume one of ten possible conformations, to which conformational energy, degree of degeneracy and area per chain is attributed [19]. Total energy of the system is calculated as a sum of van der Waals interactions between chains, conformational and surface energies. In the Pink model, the structure of lipid polar

heads is not considered and the surface energy term represents the dipolar interactions responsible for the membrane integrity. This approach provided good models of the gel–fluid transition but it does not allow for studying the influence of ionic strength on lipid membrane. The study 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 [13].

2. Method

An influence of ionic strength on membrane energetic and conformational state, which corresponds to membrane stability, is studied by Monte Carlo method based on the model in which both hydrophobic layer of hydrocarbon chains and hydrophilic layer of polar heads contribute to the total energetic state of the membrane. The model assumptions are as follows:

- Membrane is modeled by two non-interacting hexagonal lattices, whose nodes represent acyl chains.
- Periodic conditions are imposed on the boundaries.
- Each lipid molecule includes two hydrocarbon chains and a polar head, hence one molecule sits on two neighboring nodes.
- Each chain interacts with six nearest neighbors.
- Possible conformations of a chain are divided into 10 distinct states based on their conformational energy. The number of actual conformations in each state α is represented by degeneracy D_α ranging from $D_1 = 1$ for all-trans conformation to $D_{10} = 354\,294$ for the fluid state [19].
- Chain conformation is defined by angles between C–C bonds. The angle is approximated as 35° or 145° to the bilayer normal for trans bonds and 90° for gauche bonds (Fig. 1). 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) [23].
- Fixed distance between positive charges at N atom of the choline group and negative at the P atom, $d_{\mathrm{PN}} = 5 \times 10^{-10}$ m.
- Lipid molecules can rotate of 180 degrees around normal to the membrane surface.
- Lipid heads are zwitterionic, represented as dipoles. Electrostatic interactions between them included into the model.

- Polar heads can assume one of two possible tilts toward the membrane surface 78° (standing) and 30° (lying), Fig. 1. This assumption models two extreme positions of the heads [17] (and references therein).
- Polar heads can rotate towards their nearest six neighbors (nodes).
- Interactions between dipoles are valid for longer range and next nearest neighbors are also considered. Calculations include 14 neighboring dipoles [18].

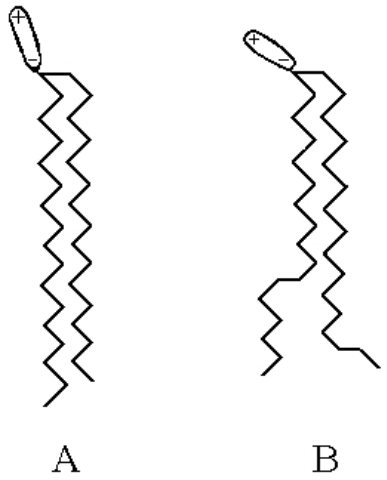

Fig. 1. Two exemplary conformations of lipid molecules. (A) Both chains assumed all-trans conformation. The angle between C–C bond to the membrane normal is 35° or 145°. The head is in the standing position here (78°). (B) Both chains have one gauche C–C bond which forms an angle 90° with the bilayer normal. For the contrast, the head is in the lying position (30°).

Hamiltonian of the studied system involves 3 terms — energy of van der Waals interactions H_{vdW}, conformational energy H_{conf}, and energy of electrostatic interactions between polar heads H_{dip} [19].

$$H = H_{\mathrm{vdW}} + H_{\mathrm{conf}} + H_{\mathrm{dip}}\,, \tag{1}$$

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

J_0^M denotes interaction energy between two parallel chains in all-trans conformation. Lattice co-ordinates are i (site index ranging from 1 to N) and j (index of 6 sites neighboring with site i). Index of chain conformational

state n and chain conformational state m range from 1 (all-trans) to 10 (fluid) [12]. A distance r_{ij} between 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 acyl chain in conformation n yields

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

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

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

p is the index of C–C bond in the chain.

The bond is characterized by 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 van der Waals interactions between 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 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 fluid state and $w_n = 1$ if $n \neq 10$. The factor was introduced by Mouritsen [19] in the modification of Pink's model to provide good agreement between the model and experimental data.

Conformational energy H_{conf} is defined based on the predefined [19] values of the chain internal energies E_n

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

Interactions between polar heads H_{dip} are due to electrostatic interactions

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

where ε is electrolyte dielectric constant, ε_0 permittivity constant, $Q_{\alpha i}$ an effective polar head charge. $Q_{\alpha i} = q/2$, where q is the actual dipole charge which equals an elementary charge [17] 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 valency of polar head, F is Faraday constant, T temperature, c ionic strength of the solution and R is gas constant.

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. The canonical ensemble was assumed. The system was equilibrated for 1000 Monte Carlo steps per site, then 10000 steps per site were performed. A series of microconfigurations, which is a Markov process, was selected by means of Metropolis method [19].

3. Results and discussion

The model tested an influence of ionic strength on energetic state of the membrane, interactions between molecules and configuration of polar heads. The membrane was tested at the gel temperature T= 300 K. The dielectric constant of the electrolyte in the proximity of lipid heads was assumed $\varepsilon = 40$, which is the optimal value to simulate the physical properties of the membrane in this model [18]. A mechanism responsible for increased durability of lipid membrane to electroporation in higher ionic strength was investigated.

The total energy H of the membrane proves sensitive to the ionic strength (Fig. 2) assuming more negative values as the ionic strength increased. This result stands for better stability of the membrane, which is observed experimentally [4, 5]. Three components contribute to the value of total energy — energy of electrostatic interactions between polar heads H_{dip}, energy of van der Waals interactions between acyl chains H_{vdW} and configurational energy of the chains H_{conf}. As expected, the change in the value H_{dip} reveals the screening effect from higher ionic concentration (Fig. 3, solid line). Positive value of the energy, representing repulsion between dipoles, decreases of approximately 6×10^{-20} J, while the ionic concentration rises from 10 mM to 3000 mM. However the change of interactions is lower than expected (Fig. 3, dashed line). It can be explained by closer packing of the molecules. The reduced repulsion in hydrophylic part allows for smaller distances between chains. This, in turn, influences the energetic state of the hydrophobic part and leads to an increase in attracting van der Waals

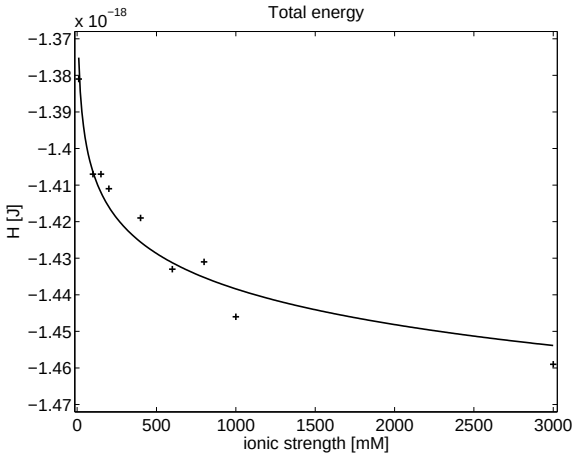

Fig. 2. Total energy H of the membrane assumes more negative values as ionic strength is increased. The membrane accepts energetically more stable configuration in higher ionic concentration.

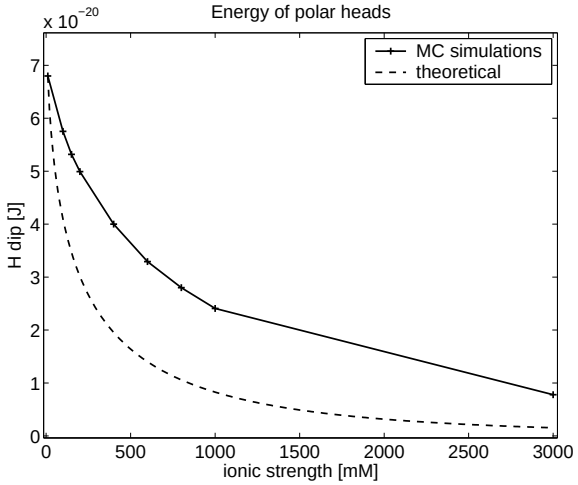

Fig. 3. Energy of interactions between polar heads H_{dip} decreases due to the screening effect from ions in the solution (solid line). The result compared to the theoretical dependence (dashed line) when head–head distances assumed constant.

interactions of about 1×10^{-20} J (Fig. 4). Therefore, the molecules from the membrane are more stable, which is caused both by the change in interactions of hydrophylic and hydrophobic part. Except distances, the chains change also their conformation, which can be seen from H_{conf} value (Fig. 5). A decrease of the conformational energy of about 0.5×10^{-20} J reflects an in-

creased number of straighten chains with lower degeneracy factor. It means that statistically more molecules are in a gel state improving stability of the bilayer in the given temperature 300 K.

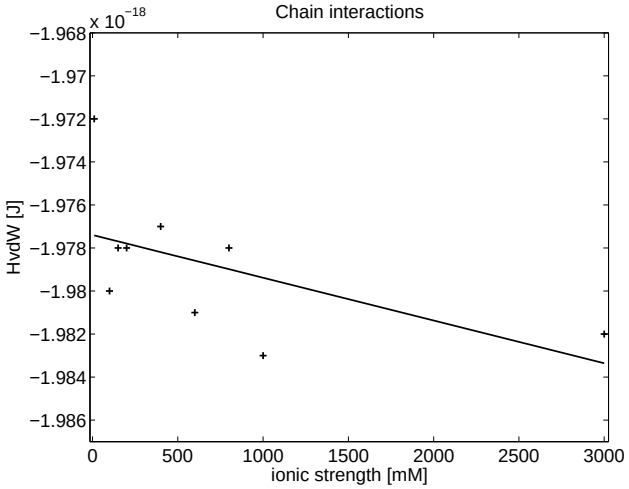

Fig. 4. Energy of Van der Waals interactions H_{vdW} between acyl chains. Certain decrease observed, which results from smaller distances between chains, and contributes to tighter binding inside the hydrophobic layer.

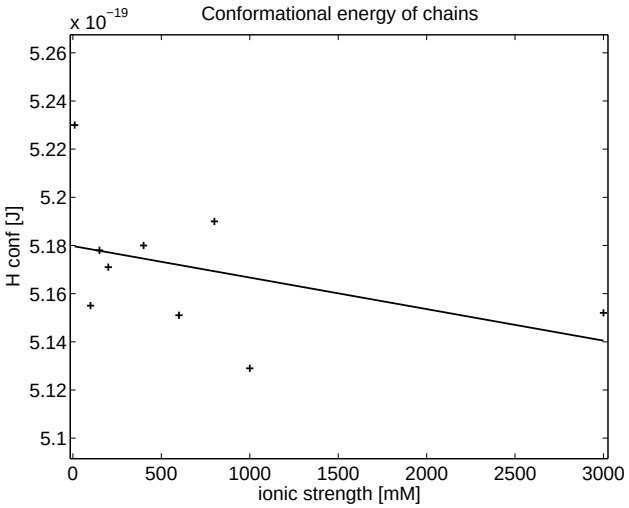

Fig. 5. Conformational energy of acyl chains H_{conf} shows slight decrease, which may indicate that acyl chains are more stable and fewer molecules accept the fluid state.

An unexpected and very interesting effect was observed in the configuration of the polar heads. The tilt of the dipoles shows sensitivity to ionic strength (Fig. 6). At low values of ionic strength (10 mM) only 25% of polar heads assume the standing configuration (78°). This number increases 2-fold at 3000 mM. A major consequence of this phenomenon could be higher accessibility of the membrane to ions from the solution and their subsequent binding. It may concern sodium or chloride ions, as well as other molecules or compounds present in the solution, changing membrane properties.

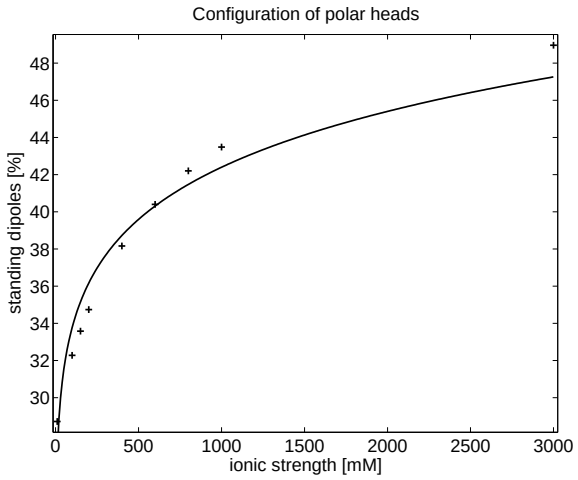

Fig. 6. The number of standing dipoles significantly increases with the ionic strength. This result may indicate higher accessibility for ions from the solution.

Facilitated penetration of sodium ions to the membrane hydrophylic part and their consequent binding to the carbonyl oxygens in polar parts of lipids was reported by Böckmann *et al.* [9] based on molecular dynamics simulations for palmitoyl-oleoyl-phosphatidylcholine (POPC) membrane. As an effect larger complexes with reduced mobility form, which may result in decreased diffusion coefficient. The hypothesis is in accordance with experimental results showing the dependence of the diffusion coefficient on NaCl concentration. For POPC membranes the lateral diffusion coefficient was measured as 6.5×10^{-8} cm^2/s in the absence of NaCl, and decreased to 1.1×10^{-8} cm^2/s at 110 mM concentration of NaCl [24]. Lower mobility of the molecules can additionally contribute to the elevated stability of the membrane in high electric field.

The MC simulations presented here show that binding monovalent ions such as sodium ions and a consequent decrease in the diffusion coefficient may originate from more favorable energetic state of the membrane in which lipid polar heads are more likely to assume the protruding configuration with the tilt 78°.

4. Conclusions

The Monte Carlo simulations based on modified Pink model proved the influence of ionic strength on configuration and interactions between lipid molecules in bilayer in the gel state, which has an effect on membrane integrity and stability. We searched for the mechanism responsible for the decreased sensitivity of lipid membranes to electroporation in higher ionic strength. The question was if this effect is only due to the screening effect of the electrolyte, diminishing repulsive forces between polar parts of lipid molecules, or there are other contributing factors. The simulations showed that the membrane becomes more stable in higher ionic strength, assuming more negative value of the total energy. The most significant change comes from lower electrostatic interactions between polar heads. However, the change of interactions is lower than expected due to the closer packing of the molecules. On the other hand van der Waals interactions between hydrophobic chains also increase binding the internal part of the membrane more strongly. The effect on chain configuration was observed, too. Acyl chains tend to assume more energetically stable conformation with decreased degeneracy coefficient.

Additionally, the effect of ionic strength on polar heads configuration was revealed. The number of standing dipoles with the tilt 78° increases twofold while comparing 10 mM and 3000 mM solutions. This result means that accessibility of ions from the solution rises in higher ionic strength. It may involve sodium or chloride ions, as well as other molecules or compounds present in the solution, and change membrane properties. The finding is in accordance with MD simulations showing facilitated penetration of sodium ions into the membrane and their binding to the carbonyl oxygens in polar parts. Larger complexes that form as a result have lower mobility, which may account for the experimentally observed decrease of the lateral diffusion coefficient in higher ionic strength. We conclude that the primary source of this phenomenon is the energetic state of the membrane, more favorable for higher rate of standing polar heads.

REFERENCES

[1] S. Koronkiewicz, S. Kalinowski, K. Bryl, *Biochim. Biophys. Acta* **1510**, 300 (2001).

[2] S. Koronkiewicz, S. Kalinowski, K. Bryl, *Biochim. Biophys. Acta* **1561**, 223 (2002).

[3] S. Koronkiewicz, S. Kalinowski, *Biochim. Biophys. Acta* **1661**, 196 (2004).

[4] M. Kotulska, S. Koronkiewicz, S. Kalinowski, *Acta Phys. Pol. B* **33**, 1115 (2002).

[5] M. Kotulska, S. Koronkiewicz, S. Kalinowski, *Phys. Rev.* **E69**, 0319 (2004).

[6] A. Chang, C. Donald, *Guide to Electroporation and Electrofusion*, Academic Press, 1992.

[7] F. Aguel, K.A. Debruin *et al.*, *J. Cardiovasc. Electrophysiol.* **10**, 701 (1999).

[8] A. Al-Khadra, V. Nikolski, I.R. Efimov, *Circ. Res.* **87**, 797 (2000).

[9] R.A. Böckman, A. Hac, T. Heimburg, H. Grubmüller, *Biophys. J.* **85**, 1 (2003).

[10] M. Langner, H. Pruchnik, K. Kubica, *Z. Naturforsch.* **C55**, 418 (2000).

[11] S.A. Tatulian, "Ionization and ion binding" in *Phospholipids Handbook*, Ed. G. Cevc, Marcel Dekker, New York (1993), p. 511.

[12] D.A. Pink, T.J. Green, D. Chapman, *Biochemistry* **19**, 349 (1980).

[13] K. Kubica, *Cell. Mol. Biol. Lett.* **2**, 257 (1997).

[14] K. Kubica, *App. Math. Comput.* **87**, 261 (1997).

[15] K. Kubica, *Task Quarterly* **2**, 601 (1998).

[16] J. Sarapuk, K. Kubica, *Cell. Mol. Biol. Lett.* **3**, 261 (1998).

[17] K. Kubica, *Comput. Chem.* **25**, 245 (2001).

[18] K. Kubica, *Comput. Chem.* **26**, 351 (2002).

[19] O.G. Mouritsen, A. Boothroyd, R. Harris, N. Jan, T. Lookman, L. MacDonald, D.A. Pink, M.J. Zuckermann, *J. Chem. Phys.* **79**, 2027 (1983).

[20] J.H. Ipsen, O.G. Mouritsen, M. Bloom, *Biophys. J.* **57**, 405 (1990).

[21] M.M. Sperotto, O.G. Mouritsen, *Biophys. J.* **59**, 261 (1991).

[22] W. Okulski, *Acta Societatis Botanicorum Poloniae* **65**, 257 (1996).

[23] H. Hauser, *J. Mol. Biol.* **137**, 249 (1980).

[24] J. Seelig, A. Seelig, *Q. Rev. Biophys.* **13**, 19 (1980).