DISTRIBUTION OF DEFECTS IN LIPID MEMBRANES. COMPUTER SIMULATION

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ABSTRACT

Based on the Monte-Carlo method, the ten-state Pink model of lipid membrane was completed by the dynamic Kawasaki-type processes of nearest neighbour exchanges of lipid molecules and vacancies in lipid chain lattice. The influence of the number and distribution of vacancies in membrane on its permeability was studied. The snapshots of microconfigurations of the dipalmitoylphosphatidylcholine (DPPC) membrane model obtained from Monte-Carlo simulation were analyzed. Temperature dependences of the area of vacancies in the membrane and regional probability of an ion transfer through interface region were determined.

KEY WORDS: lipid membrane, phase transition, pores, passive permeability, computer simulation.

INTRODUCTION

Pure lipid membranes, i.e. lipid bilayers and lipid monolayers, are examined as important models of biological membranes. Lipid bilayers are used to study such membrane phenomena as the main phase transition, passive transport of ions and molecules, effect of intrinsic molecules on membrane parameters.

Pure lipid bilayers are almost impermeable to ions except the phase-transition region in which the permeability rapidly increases (Papahadjopoulos, Jacobsen, Nir and Isac 1973). In recent years, a number of theoretical papers have appeared to explain this phenomenon. According to Papahadjopoulos et al. (1973), Cruzeiro-Hansson et al. (1988) and Mouritsen (1990) this enhancement of passive ion permeability can be related to the area of interfacial regions separating the gel and fluid domains (clusters) in either monolayer of the membrane. The authors of the second, above mentioned, paper suggested that the interfacial regions could contain the structural defects because of the mismatch in molecular packing, which was responsible for the leakiness of membrane near the phase transition.

This conception was amplified in the paper by Cieniawa and Gawda (1993). Using the algorithm of the Monte-Carlo method proposed earlier by Mouritsen (1990), the authors of paper (Cieniawa et al. 1993) caused a lattice of lipid chains achieve the state of thermodynamic equilibrium. Afterwards they analysed the number of sites in lattice undergoing strong lateral fluctuation of density. From such an analysis Cieniawa et al. (1993) supposed, in contradiction to the suggestion of Cruzeiro-Hansson et al. (1988), that membrane permeability did not depend on the total size of interfacial region because that region was not all occupied by vacancies (also called defects or holes). Nevertheless, the model used for computer

simulation neither by Cruzeiro-Hansson et al. (1988) nor by Cieniawa et al. (1993), gave entrance to explicit existence of vacancies in the lattice of lipids.

The conception of defects as holes in membrane lattice of lipids is similar to the one described by Gordeliy, Ivkov, Ostanevich and Yaguzhinskij (1991). They examined D₂O-contrasted defects in DPPC multilayers by a small-angle neutron scattering method. To explain results of experiment, Gordeliy et al. (1991) proposed an existence of the annular defects between adjacent clusters because of non parallel attitude of the all-trans chain segments adjacent to the polar head group regions.

The lipid monolayer permeability was also studied theoretically by cellular-automaton method (Kubica and Kuczera 1990, 1991). Presented in the former paper, conception of pores in lipid monolayer as complexes of vacancies in a model lattice is similar to that used in this paper. However, the temperature dependence of the lipid monolayer permeability near the phase transition temperature is not the subject of the paper Kubica et al. 1990.

According to Kubica et al. 1991, the lipid monolayer permeability was assumed to be determined by the surface of so called "gauche blocks" i.e. areas of smaller packing density of lipid chains. These areas correspond to, presented in further chapters, clusters of chains in fluid state. The other assumption is proposed below, that the lipid monolayer permeability is proportional to the percentage of vacancies in a lattice of lipids, including vacancies in the gel and interfacial areas.

The purpose of this paper is to study the number and distribution of vacancies (holes) in the model lipid monolayer. The used model of lipid monolayer, being based on the Monte-Carlo method, is the ten-state Pink model of chain lattice completed by the dynamic Kawasaki-type process of the nearest neighbour exchange of lipid molecules and vacancies.

The model is described in the next section. The last section contains results of calculations and discussion.

MODEL OF LIPID MEMBRANE

Presented below numerical calculations are based on the ten-state model of Pink and collaborators (see e.g. Pink, Lookman, Macdonald, Zuckermann, Jan 1982 and Mouritsen 1990). This model has been very successful in describing phase transition both in pure lipid membranes and membranes with the intrinsic molecules like cholesterol or proteins. Within the "classical" Pink-Mouritsen model, the interactions between lipid monolayers in a bilayer are ignored. The translational lipid degrees of freedom are neglected. Each monolayer is represented by a two-dimensional triangular lattice. Each site of the lattice is occupied by an acyl chain. According to this "classical" model, the two chains of the lipid molecule are assumed to be independent. Each chain can exist in one of ten states: the all-trans ground state (the 1st state); eight intermediate states (2..9), which are low-energy excitations of the all-trans state; the 10-th high-energy "melted" state, which is a component of the high-temperature fluid phase. The states (2..9) are characteristic of the high-temperature gel phase. All the states of a chain α are described by the conformational energy E_α , area A_α and a degeneracy D_α .

The Hamiltonian model of a lipid monolayer consists of three contributions (Mouritsen 1990, Pink et al. 1982):

$$H = H_1 + H_2 + H_3. \quad (1)$$

The first one is the single-chain contribution:

$$H_1 = \sum_{i=1}^N \sum_{\alpha=1}^{10} E_\alpha L_{\alpha i}, \quad (2)$$

where N is the number of chains and $L_{\alpha i} = 1$, when the chain i is in the state α , otherwise $L_{\alpha i} = 0$.

The second contribution H_2 describes the interaction between chains:

$$H_2 = -\frac{J}{2} \sum_{i,j=1}^N \sum_{\alpha,\beta=1}^{10} I_\alpha I_\beta L_{\alpha i} L_{\beta j}. \quad (3)$$

Here J is the interaction strength between two nearest neighbour all-trans chains, I_α and I_β describe the quadrupole-quadrupole interaction between two nearest neighbour chains in states α and β . The summation in Eq. (3) is over nearest neighbours only. The third contribution of the Hamiltonian, H_3 , is the energy of the interaction between polar parts of lipid molecules.

$$H_3 = -\frac{K}{2} \sum_{i,j=1}^N \sum_{\alpha,\beta=1}^{10} A_1^{1/2} A_\alpha^{-1/4} A_\beta^{-1/4} L_{\alpha i} L_{\beta j}, \quad (4)$$

where K is the interaction constant.

In the present paper, the above described "classical" model was modified as follows. (i) Vacancies (holes) in the lipid chain lattice were introduced. (ii) The lateral movement of lipid molecules was permitted. (iii) The whole diacyl lipid molecules were submitted to change a position in a lattice.

Respecting (i), the holes were created as follows. First, the lattice of lipids (without holes) had achieved the equilibrium in a given temperature after 1000 Monte Carlo steps per site. Afterwards, the holes were created in sites, where in the site neighbourhood there was at least one chain in the 1st state,

and on the opposite side – the chain in the 10th state. The strong density fluctuations were expected to exist in these sites of lattice.

Subsequently, each chain in lattice was attributed to a lipid head, together with a randomly selected chain of its neighbourhood. The attribution allowed only collective translocation of the couple of chains. The procedure of the chain attribution to lipid heads left the chains for which there were not "free" chains to couple in their neighbourhood. These chains were changed into holes called "additional" holes. The procedure was repeated 100 times to achieve the minimum of the additional hole number.

Regarding (ii), the rules of the movement of lipid molecules were as follows. Finding the hole in a succeeding Monte-Carlo step, all possible dislocations of neighbouring molecules were considered and for each dislocation the change of microstate free energy ΔE was calculated. Displacement was accepted, when the lowest E had been attained, on condition that $\exp(-\Delta E/k_B T) > \xi$, where $\xi \in [0,1]$ is random number, k_B denotes the Boltzmann constant and T is a temperature.

Computer simulation was performed for the system containing 5000 molecules (10000 acyl chains) of DPPC. Periodic boundary conditions were assumed. To obtain thermodynamic equilibrium, 1500 Monte-Carlo steps per site were realized. We chose $K = 0.73 \cdot 10^{-20}$ J and adjusted J so that the temperature of the phase transition was equal 314K.

RESULTS AND DISCUSSION

In Fig. 1 there are shown three snapshots of microconfigurations of the chain lattice for temperatures below, at and above the gel-to-fluid phase transition temperature T_m equal for DPPC 314K. The figure differentiates chains in the gel state i.e. the all-trans state or eight intermediate states, and chains in the fluid state. One can see, below T_m , the clusters of the chains in fluid state in „a see” of gel phase and, above T_m , clusters of the gel phase in „a see” of fluid phase. Holes can be found in the interface regions surrounding clusters, likewise somewhere else. Some holes create complexes called pores.

The following conclusions are based on computer analyses of snapshots of chain lattice for various temperatures. Temperature dependence of the ratio between area of holes and membrane area is presented in Fig. 2. The ratio originating from additional holes is also marked. Fig. 2 indicates that the fraction of membrane area occupied by holes achieves maximum near $T_m=314$ K. If we assume that probability of a small ion crossing the lipid bilayer is determined by the square of this fraction, the maximum of the dependence shown in Fig. 2 will correspond to the maximum of membrane permeability at the phase transition temperature. The area of additional holes is insignificant and equal about 0.5% of the membrane area. The dependence presented in Fig. 2 is similar to the one shown in the paper by Cieniawa et al. (1993), where, however, the lipid translational degrees of freedom have been neglected.

The fraction of DPPC bilayer area occupied by defects at $T=328$ K was experimentally estimated by Gordeliy et al. (1991) as 8.8% or $4.8 \div 6.4\%$ taking into account the partial screening of defects by lipid head groups. The value of the fraction calculated at this temperature on the basis of our model is equal 3.7%.

Fig. 3 shows temperature dependence of the ratio between the number of holes in the interfacial region and the total hole

Fig. 1. Snapshots of microconfigurations of lipid lattice for three temperatures around the gel-to-fluid phase transition, $T_m=314$ K. For clarity only a part of the lattice is shown. The white circles denote acyl chains in the fluid states; the black circles represent chains in the gel state; dots indicate vacancies in the lattice (holes). a) $T=312$ K, b) $T=314$ K, c) $T=316$ K. Short lines connect chains of a diacyl lipid molecules.

Fig. 2. Temperature dependence of the ratio between the area of holes and membrane area (o) and the ratio originating from additional holes (+). The latter curve is shifted upwards by 5%.

number. The interfacial region is defined as the first layer of lipid chains surrounding clusters. It can be seen that the percentage of holes in the interfacial region reveals a maximum

Fig. 3. Temperature dependence of the ratio between the number of holes in interfacial regions and the total hole number.

Fig. 4. Temperature dependence of the ratio between the area of holes in interfacial regions and total area of these regions.

at T_m and rapidly decreases above T_m . This result confirms the Cruzeiro-Hansson et al. hypothesis, that the interfacial regions are responsible for the leakiness of membrane at its phase transition.

Fig. 4 presents temperature dependence of the ratio between the area of holes in interfacial regions and total area of these regions. The ratio seems to be equal to the interfacial regional probability of an ion crossing the membrane (p_i). Below T_m the percentage of hole area in the interfacial region weakly decreases when the temperature increases and is in the range of 15-25%. It would be consistent with the assumption presented in the paper by Cruzeiro-Hansson et al. (1988), that p_i is temperature-independent, but the value of p_i equal about 0.2 is significantly smaller in comparison with $p_i \approx 1$ assumed in this paper.

The ratio, as shown in Fig. 4, achieves its maximum near T_m and decreases significantly above T_m . The temperature dependence of p_i was proposed earlier as exponential in the paper of Kanehisa and Tsong (1978). The dependence showed in Fig. 4 is not consistent with this assumption. However, it should be noticed that the presented model does not take into consideration local changes of defect translocation due to the influence of an ion approaching membrane surface.

As one can see from Fig. 1, some holes create complexes called pores. Our calculations indicate, that the tendency of holes to group into pores decreases when the temperature increases.

CONCLUSIONS

Based on the Monte-Carlo method, the ten-state Pink model of lipid membrane, completed by the existence of holes in lipid chain lattice, is presented. The model allows the lipid molecules from the neighbourhood of the hole to move and occupy this empty site of lattice. Calculation results are consistent with the experimentally determined increase of lipid membrane permeability at the main phase transition. The hypothesis of Cruzeiro-Hansson et al. (1988), that the interfacial region is responsible for the leakiness of the membrane during its phase transition is confirmed. The calculated temperature dependence of the interfacial regional probability of an ion transfer differs from the dependences proposed earlier in other papers.

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ROZMIESZCZENIE DEFEKTÓW W BŁONIE LIPIDOWEJ. SYMULACJA KOMPUTEROWA

STRESZCZENIE

Oparty na metodzie Monte-Carlo, dziesięciostanowy model Pinka błony lipidowej został uzupełniony o procesy wymiany miejscami cząsteczek lipidów oraz wakansów w sieci łańcuchów lipidowych (procesy Kawasaki). Badany był wpływ liczby i rozmieszczenia wakansów w błonie na jej przepuszczalność. Analizowano uzyskane z symulacji Monte-Carlo komputerowe obrazy modelu błony dipalmitoylofosfatydylocholino (DPPC). Określono temperaturowe zależności powierzchni wakansów w błonie oraz prawdopodobieństwa przejścia jonu przez obszar styku faz.

SŁOWA KLUCZOWE: błona lipidowa, przejście fazowe, pory, przepuszczalność bierna, symulacja komputerowa.