

Received 22 July 2002
Accepted 26 August 2002

COMPUTER SIMULATION STUDIES ON THE SIGNIFICANCE OF LIPID POLAR HEAD CHARGE

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Abstract: Ripple phase modelling was achievable by taking into consideration the dipole structure of the polar heads of model membrane molecules. Computer simulations enabled the selective analysis of a model membrane. Considering only the hydrophobic part of the lipid membrane, the gel-fluid transition stage can be obtained in such a simulation. Assuming an additional degree of freedom, the entire molecule can move along the normal to the membrane surface projected from two C-C bonds. The amounts of shifted lipids were 17% and 33% at temperatures of 300 K (gel) and 330 K (fluid), respectively. Taking into account only polar head interactions in media of different ionic strength I , dielectric constant ϵ , and an effective charge and temperature, we could observe the same behaviour of the examined system independently of the values of I and ϵ when the charge was reduced to $q/2$. The amount of shifted heads at 300 K decreases sharply with the reduced charge value, with an accompanying increase in the number of “standing” polar heads. Summing up, it can be stated that hydrocarbon lipid chains exhibit a greater tendency to displacement in the fluid state than in the gel state. However, the polar heads behave in the opposite way: there are more displaced heads at 300 K than at 330 K. Thus, the overall analysis of the interactions between the molecules of the model membrane should enable us to find model parameters suitable for studying the lipid membrane at a wide range of temperatures. Finally, an electrostatic profile close to the membrane surface could be estimated in different membrane states. This should be useful in membrane-biologically active compound interaction analysis.

Key Words: Lipid Head Charge, Lipid Layer, Dipole Matrix, Monte Carlo Simulation

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INTRODUCTION

The Monte Carlo simulation, along with other simulation techniques, provides a very useful tool for examining complex systems such as biological membranes. The models created can be compared with experimental facts, making it possible to formulate criteria for choosing the proper model for a given experiment. This technique allows for the analysis of interactions between membranes and some compounds, explaining the functioning of biologically-active compounds. It may also help in establishing methods to diminish the activity of harmful compounds [1, 2]. A promising aspect of this research may be the ability to predict characteristic features of those molecules that are designed to perform certain activities in biological membranes. Moreover, on the basis of this technique, it will be possible to estimate the value of the partition factor [3], indicating effective incorporation of molecules into the membrane. Following the publication of the Pinks model [4], a number of papers have appeared making use of the model in Monte Carlo simulations of lipid membranes [5-10]. These simulations are based on the following assumptions:

The lipid membrane is modelled as a triangular lattice.

- Ø Each site of the triangular lattice is occupied by a hydrocarbon chain.
- Ø Each hydrocarbon chain takes one of the ten possible conformations.
- Ø Lipid chains are treated separately.
- Ø The conformations are described by internal energy, degeneracy and area per chain.
- Ø The energy of the membrane consists of van der Waals, conformational and surface energies (the structure of the polar heads is not considered).

Pinks' model was used to analyse the behaviour of lipid molecules in the membrane, with a detailed description of the alkyl chains only. It gives a good description of the gel-fluid transition that makes allowance for the influence of the chain lengths of some amphiphilic compounds on membrane properties [1, 2, 5, 7, 8]. However, the model did not consider the details of the polar head structure of lipid molecules. Therefore, it was not possible to involve parameters of the medium such as ionic strength, pH, or other factors interacting with the membrane via the polar part.

The most important reason why the lipid polar head structure should be taken into consideration in model research are the facts proving that lipids are not only passive membrane compounds, and that the lipid bilayer should be recognized as a potent enhancer and regulator of surface-associated reactions [11-13]. Electrostatic interactions seem to be one of the most important forces in determining the way macromolecules interact with membranes. They play a role in fusion events [14], endocytosis [15], exocytosis [16], and pore and domain formation [17-18].

Recent research [19] has put particular stress on the details of the structure of the polar part in lipid molecules. New assumptions have been added to those mentioned above:

- Ø Each hydrocarbon chain has one polar part – a dipole.

- ∅ Lipid molecules are allowed to move perpendicularly to the membrane surface.
- ∅ The polar part of each molecule can change its tilt toward the membrane surface, which can take one of two values, 78° or 30°.
- ∅ Polar heat can be directed towards one of the six nearest neighbours.

The results for the system with variable temperature showed the gel-fluid transition, known of before [4], but also the occurrence of a wavy structure [19] resembling the "ripple phase", that had been experimentally observed [20]. However, the jump in the specific heat at temperatures close to gel-fluid transition was not observed. So, the next step was only concerned with the behaviour of dipoles placed in the nodes of a triangular lattice by taking the real distance that follows from the area per molecule characteristic for the gel and fluid phases. In addition, one polar head was connected with two hydrocarbon chains. The polar heads of membrane lipids are located between the hydrophilic and hydrophobic regions, where the dielectric constant changes from 2 to 80 [21]. It was proved that if the dielectric permeability of a membrane polar part is significantly smaller than water's dielectric permeability, then the membrane model does not have to take into account the changeability of the dipole tilt towards a(the) membrane surface. This assumption becomes more significant for dielectric permeabilities ϵ approaching $\epsilon = 80$. The packing degree of hydrocarbon chains in the hydrophobic part of the membrane is also responsible for the angle value between the dipoles and the membrane surface [22]. Thus, the interaction of molecules in the hydrophobic part of a membrane can influence the dipole behaviour in the hydrophilic part.

In order to consider these results further, we decided to reduce the dipole charges and examine the effect of this reduction on membrane behaviour. Such a model allows for molecular dynamic results which show that water molecules in the DPPC interface almost compensates for the PN dipole of the headgroup [23].

MODEL AND CALCULATION METHOD

The lipid membrane is modelled as two non-interacting triangular lattices. To make the model membrane a closed surface, periodic conditions are imposed in the horizontal and vertical direction. The current model's assumptions reflect the real membrane properties far better than those of the previous one [19]:

- Each molecule modelling a lipid consists of two hydrocarbon chains and a polar head – a dipole and two chains occupy two neighbouring nodes.
- The chain closer to the polar head is the shorter of the two C-C bonds, since the two carbon atoms of the β -chain extend parallel to the membrane surface [24].
- The chains can assume one of 10 distinct conformations [4].
- The distance between the charges – positive at the N-atom of the choline group and negative at the P-atom – was assumed to be 5×10^{-10} m.

- Due to phospholipid symmetry, all the molecules can rotate 180 degrees around the axis perpendicular to the membrane surface.
- The whole molecule can move along the normal to the membrane surface by a distance of the projection of two C-C bonds.
- The dipoles can assume one of two possible tilts toward the membrane surface: 78° (“standing”) and 30° (“lying”).
- Dipoles can rotate towards their nearest six neighbours.
- The distance between lattice nodes equals the average distance between the chains of the DPPC membrane at selected temperatures, 5.1 x 10⁻¹⁰ m and 6.58 x 10⁻¹⁰ m at 300 K (gel phase) and at 325 K (fluid phase), respectively.
- The dipole interaction area was extended to 14 dipoles, which also included more distant neighbours. The interaction range is presented in Fig. 1. (previously, the dipole interactions were restricted to their nearest neighbours [19]). The mentioned degrees of freedom are shown in Fig. 2.

The Hamiltonian of the studied system takes the form:

$$H = -\frac{J_0^M}{2} \sum_{\langle i, j \rangle} \sum_{n, m} I_{nm}(r_{nm}) L_{ni} L_{mj} + \sum_i \sum_n E_n L_{ni} + \frac{1}{2} \sum_i \sum_{j=1}^{14} \sum_{\alpha, \beta=-1, 1} \frac{Q_{i\alpha} Q_{j\beta} \exp(-\kappa r_{i\alpha j\beta})}{4\pi\epsilon\epsilon_0 \kappa r_{i\alpha j\beta}}$$

where:

J_0^M - the energy of the interaction between two parallel all-trans chains,

$$I_{nm}(r_{nm}) = \sum_{p, q} \frac{S_{np} S_{mq}}{S_0^2} f(r_{nm})$$

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

$$S_0 = \sum_p S_{Gp}$$

$$f(r_{nm}) = \left(\frac{r_0}{r_{nm}} \right)^5 \quad [4]$$

$$k = \sqrt{\frac{2z^2 F^2 c}{e_0 e R T}}$$

i – the number of the lattice site,

j – the number of neighbouring dipoles (Fig. 1),

ϵ – the dielectric constant,

ϵ_0 – the permittivity of free space,

$Q_{i\alpha} = 1$ or -1 for $\alpha = 1$ and $\alpha = -1$ respectively (the same is valid for the β index),

$r_{i\alpha j\beta}$ – the distance between two charges, α and β , of the dipoles at sites i and j ,

κ – the inverse of the Debye length,

z – the valency,

F – the Faraday constant,

R – the gas constant,

T – temperature,
 c – concentration,
 L_{ni} – the lipid chain projection operator for lattice site i and lipid chain state n ,
 $\langle i,j \rangle$ – the sum over the nearest -neighbour sites,
 r_{nm} – the distance between two chains in states n and m at sites i and j ,
 E_n – the internal energy of a chain in state n ,
 S_{np} – the order parameter of the p^{th} C-C bond,
 Θ_{np} – the angle that this bond makes with the normal to the bilayer,
 r_{ij} – the distance between two charges at sites i and j .

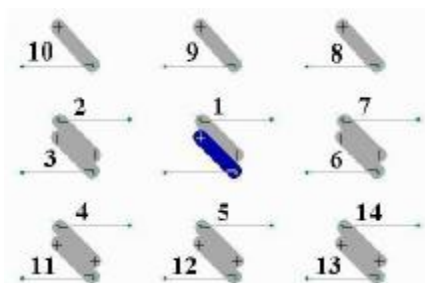


Fig.1. Range of interactions between dipoles. Each dipole interacts with 14 other dipoles: nearest neighbours (lipids), numbers 1 to 6, and 8 more distant neighbours, numbers 7 to 14.

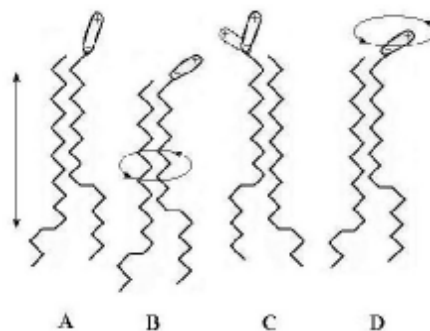


Fig. 2. Model degrees of freedom. A – rotation of the whole molecule ($\pm 180^\circ$), B – molecule displacement by a projection distance of two C-C bonds, C – dipole rotation towards 6 nearest neighbours, D – dipole can assume one of two possible tilts: 78° (“standing”) and 30° (“lying”).

The presented Hamiltonian, as well as the van der Waals interactions and conformational energy, describes the screened electrostatic interactions with the second neighbour's cut-off. Because of the second neighbour's area symmetry (Fig. 1), the variation of the dipoles' orientation and tilt angles changes the cut-

off value is not constant; its maximal value is approximately 2.6×10^{-9} m at 300K (gel) and 3.14×10^{-9} m at 325 K (fluid). Such a cut-off, greater than 1.8×10^{-9} m, taken for the electrostatic interaction of neutral lipids appears to work well for PC lipids [25]. Due to the charge screening, the model is sensitive to environmental conditions such as ionic strength and dielectric permeability. The simulations were performed in a canonical ensemble. In this ensemble, the system is coupled to a heated bath that provides the energy needed to establish the equilibrium state. To generate a series of micro-configurations, a Markov process should be constructed in such a way that the limit distribution will correspond to the equilibrium distribution in the canonical ensemble. The Metropolis method, which was employed here, involved the following steps:

1. Initial configuration determination.
2. Random generation of a new configuration.
3. Evaluation of the energy change $\Delta H = H_{\text{new}} - H_{\text{old}}$.
4. If $\Delta H < 0$, then the new configuration is accepted (return to step 2).
5. If $\exp(-\Delta H/kT) >$ random number from the range $[0, 1]$, then the new configuration is accepted. Otherwise return to step 2.

In each step of the computer simulation, the chosen particle was randomly attributed with: a shift of the whole particle into the membrane interior (of value 2.6×10^{-10} m) or its absence, a polar head tilt (78° or 30°), and a polar head orientation (towards one of the six nearest neighbours). Moreover, the whole molecule can rotate 180° (Fig. 2).

The studied lattice included 100 nodes (50 dipoles). The system was equilibrated for 1000 Monte Carlo steps per site in the given temperature; then 10,000 steps per site were performed. Simulations were performed at 300 K and 325 K. The average distances between lattice nodes was assumed to be 5.1×10^{-10} m (gel phase) and 6.58×10^{-10} m (fluid phase).

RESULTS AND DISCUSSION

Tables 1 and 2 present lipid polar head (dipoles) interaction energies. The dipoles are located in a triangular 10×10 node lattice, and their behaviours are considered at 300 K and 330 K, which correspond with the gel and fluid phases, respectively. Below each energy value, expressed in Joules, there are percentage amounts of shifted, and in brackets, "standing" dipoles. The values (collected in the tables) have been calculated for different dielectric constant ϵ values (80 and 40), ionic strengths I (10 and 100 mM) and effective dipole charges: q , $q/2$, $q/4$ and $q/8$, where q is an elementary charge. All the presented values average 100,000 Monte Carlo steps per lattice node.

An analysis of dipole system interaction energies shows negative energy values irrespective of ϵ , I or effective charge q values at 300K, although they rise with decreasing q and with increasing I and ϵ . At 330 K, when the intermolecular distance is characteristic for the fluid phase, the energy of the dipole interaction is negative, but only for the whole (elementary charge) dipole charges.

Tab. 1. Polar head interaction energies [J] and percent amount of shifted (“standing”) dipoles. All values have been calculated at 300 K, for different dielectric constant ϵ values (80 and 40), ionic strengths I (10 and 100 mM) and effective dipole charges: q, q/2, q/4 and q/8.

T = 300K				
$\epsilon = 80$		$\epsilon = 40$		
I = 10mol/m ³	I = 100 mol/m ³	I = 10mol/m ³	I = 100 mol/m ³	
-1.63x10 ⁻¹⁸	-1.59x10 ⁻¹⁸	-3.31x10 ⁻¹⁸	-3.06x10 ⁻¹⁸	q
49.0(24.8)	51.0(23.6)	50.0(0)	52.1(0)	
-1.70x10 ⁻¹⁹	-1.67x10 ⁻¹⁹	-6.58x10 ⁻¹⁹	-6.25x10 ⁻¹⁹	q/2
51.4(28)	51.1(28.2)	49.5(7.3)	49.7(8.2)	
-1.27x10 ⁻²⁰	-1.29x10 ⁻²⁰	-4.19x10 ⁻²⁰	-4.04x10 ⁻²⁰	q/4
51.5(46.4)	51.3(45.8)	51.9(41.0)	51.8(41.8)	
-1.88x10 ⁻²¹	-1.97x10 ⁻²¹	-4.76x10 ⁻²¹	-4.63x10 ⁻²¹	q/8
50.0(49.6)	50.3(49.1)	50.9(48.2)	50.7(48.5)	

Tab. 2. Polar head interaction energies [J] and percent amount of shifted (“standing”) dipoles. All values have been calculated at 330 K, for different dielectric constant ϵ values (80 and 40), ionic strengths I (10 and 100 mM) and effective dipole charges: q, q/2, q/4 and q/8.

T = 330K				
$\epsilon = 80$		$\epsilon = 40$		
I = 10mol/m ³	I = 100 mol/m ³	I = 10mol/m ³	I = 100 mol/m ³	
-1.54x10 ⁻¹⁹	-1.30x10 ⁻¹⁹	-1.25x10 ⁻¹⁸	-1.14x10 ⁻¹⁸	q
18.3(21.2)	16.5(23.2)	41.3(3.0)	40.0(4.2)	
3.12x10 ⁻²⁰	2.66x10 ⁻²⁰	3.21x10 ⁻²⁰	2.76x10 ⁻²⁰	q/2
2.6(43.4)	2.5(44.3)	5.3(36.6)	4.5(39.5)	
9.64x10 ⁻²¹	8.27x10 ⁻²¹	1.81x10 ⁻²⁰	1.36x10 ⁻²⁰	q/4
1.3(47.8)	1.4(48.6)	1.5(47.2)	1.6(47.5)	
2.51x10 ⁻²¹	2.16x10 ⁻²¹	4.87x10 ⁻²¹	3.70x10 ⁻²¹	q/8
1.3(49.5)	1.3(49.9)	1.4(49.1)	1.3(49.3)	

Decreasing the effective dipole charges, we can observe positive interaction energies. Negative and positive energy values describe the attraction and repulsion of a dipole system. Lateral head repulsion and chain attraction cause packing frustration with a resulting ripple phase appearance [26]. It is worth

examining the configuration of a set of whole lipids (head and chains), for a chosen value of ϵ and I , and a reduced dipole charge, to find the temperature at which the sign of the head interaction energy changes. Moreover, bearing in mind the change in the energy sign with an effective charge at 330 K, we can expect that at pretransition, a change in membrane hydration takes place due to water order variation, which influences the lipid dipole compensation.

In order to minimize the ability to force some of the molecular configurations, we assumed that the lipids may displace with respect to the membrane normal. Computer simulation results show that for some values of the model parameters, the examined system loses the mentioned ability. Considering the amount of shifted dipoles at 300 K, we observed that about 50% of the dipoles are shifted independently of ϵ and I or the effective charge. Above the gel-fluid transition temperature, the amount of shifted dipoles begins to change with the mentioned parameters. The number of shifted dipoles decreases with smaller effective charges and with a greater dielectric constant. The decrease is weaker with smaller effective charges. For all the analysed parameters, the dependence of the shifted dipoles on the ionic strength is very weak.

Another aspect of the examined system is the role of hydrocarbon chains in molecular displacements. When only chains without polar heads were placed in a triangular lattice, it was observed that about 17 % of these chains shifted at 300K, and at 330 K, when the chains were in a fluid state, the amount of shifted chains doubled. The all-trans conformation (300K) does not favour chain shifts, a conclusion following from the van der Waals interactions. Only the polar heads behaved differently: at 300K, they showed a greater tendency toward displacement than at 330K, where the distances are greater. Taking into account the mentioned tendencies of chains as well as those of polar heads, we could find in the first approximation a waved membrane structure with a period equal to 45 nodes before the gel-fluid transition temperature, and its disappearance above the transition temperature [19].

The next degree of freedom sensitive to the model parameters of the examined system was the polar head tilt. A change in the polar head tilt angle may have physiological significance [27]. In the literature, there are various values for the PN (of polar heads) vector inclination [19], so it was interesting to analyse the behaviour of polar heads for different environmental conditions: the dielectric constant ($\epsilon = 80$ and 40) and ionic strength (10mM and 100mM). From our model, it follows that the number of "standing" dipoles depends mainly on the temperature. At 300K, the amount of "standing" dipoles rises with a decrease in the effective charge and an increase in the dielectric constant and ionic strength values. At a higher temperature (330K), dipoles behave completely differently: the amount of "standing" dipoles rises more sharply with a smaller effective charge value. The amount of "standing" dipoles in the examined system was very sensitive to the ionic strength value $\epsilon = 40$ when the charge was reduced to half (Fig. 3).

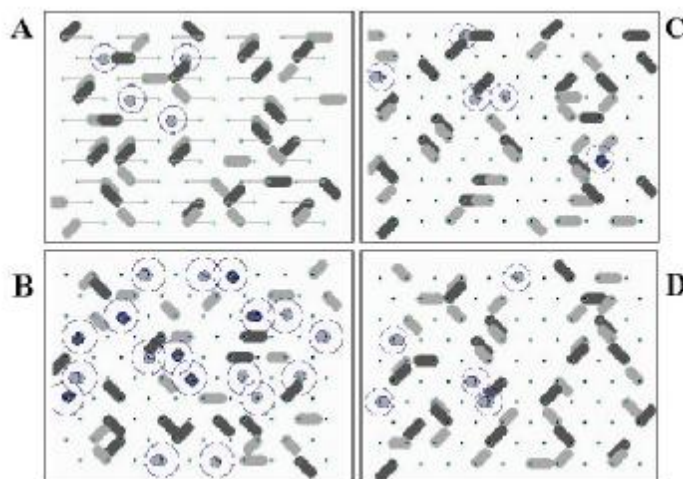


Fig. 3. Typical states of dipoles at 300 K – (A) and (B); at 330 K – (C) and (D). The ionic strength $I = 10$ mM – (A) and (C); $I = 100$ mM – (B) and (D). The dielectric constant $\epsilon = 40$, and the charge is reduced to half the elementary charge. “Lying” dipoles are long rods and “standing” dipoles are short rods. Molecules shifted down are black rods, non-shifted are grey. To make it more visible the “standing” dipoles are circled.

So, if we want to build a membrane model that is sensitive to dielectric constant and ionic strength – a model that connects changes in the hydrophobic and hydrophilic parts [22], as follows from the data in Tables 1 and 2 – the effective charge of the dipoles should be reduced to about half its elementary charge.

There have been a number of theoretical attempts to explain the experimentally observed ripple structures. The question whether the essence of the phenomenon follows from monolayer, bilayer or bilayer stack properties is still under discussion. Some attempts are based on the hydrophobic membrane part, but only treat the polar part very generally. Based on the classical Pinks model, Trandum and coworkers [28], by locally changing the value of one model parameter – (the lateral pressure), have observed a new jump in the specific heat value, corresponding to the sub-main transition (pretransition). However, as previously discussed [29], in changing the value of the mentioned parameter we can shift the main gel-fluid transition temperature. The results of the model simplifications (mainly equal lengths of the hydrocarbon chains and their molecular independence), should rather be treated as the sum of two lipid systems with different gel-fluid transition temperatures considered together. On the basis of the lipid bilayer properties, Heimburg [30] tried to explain the ripple phase phenomenon. The basis of his model is the assumption that both pretransition and main transition are caused by the same physical effect – chain melting. The membrane ripples consist of fluid lipid line defects. Kodama and Miyata [31] show that, in membranes composed of phosphatidylethanolamine

that differs from phosphatidylecholne in its polar head structure, no ripple phase was found. So, the question arises: should theoretical models exhibiting the ripple phase transition take into account the polar head structure? Moreover, the ripple phase is observed only in fully hydrated phospholipids. Thus, if water plays an essential role in this phenomenon, it follows that a hydrophilic membrane part is an important factor. There are number of papers showing the correlation between head ordering and alkyl chain conformations [32].

An example of a molecular model which contains the ripple phase transition is the model of a lamellar stack of bilayers published quite recently by Benerjee [26]. In this model, ripples appear to compensate the packing frustration between the headgroups and the hydrocarbon chains. An essential requirement for the appearance of ripple structures is the presence of large headgroups, hydration and a molecular tilt with respect to the membrane normal. Since the model has not considered the structure of the polar heads, it is not able to distinguish the differences between phophatidylcholines and phophatidyletanolamines, for which the ripple structure is not observed. However, the differences in behaviour of membranes formed from the earlier-mentioned phospholipids may follow not from the head structures but from the chain tilt differences.

Pearson and Pascher [33], studying crystalline DMPC, observed mutual 2.5×10^{-10} m displacement of the two molecules of DMPC in the direction of the hydrocarbon chain. Although such a form of DMPC is not fully hydrated, this displacement may be connected with the ripple structure. As mentioned earlier, our model, by admitting molecule displacements and treating polar heads as mobile dipoles, has also shown a waved monolayer structure. However, although the model assumptions have been improved and brought closer to reality, we have not yet managed to find a good enough set of model parameters to observe similar behaviour of lipids at different temperatures. Obviously, we could change the van der Waals constant value to that which would give the desired effect, but the gel-fluid transition temperature would become far from what is expected. So in our follow-up research, as shown earlier [34], where the interaction of both lipid layers may be important in some cases, we shall try to consider the second lipid layer.

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