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POLAR HEAD CHARGE OF MEMBRANE MODIFIERS AND THEIR BIOLOGICAL ACTIVITY: THE MONTE CARLO SIMULATION STUDIES

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Abstract: On the basis of the 10-state Pink model, the effect of the polar head charge of amphiphilic modifier molecules has been discussed. It was shown, that for small concentrations of anionic and cationic compounds inserted into a layer composed of electroneutral lipids the electrostatic interaction, in spite of the long-range character, can be limited to the nearest neighbour interaction. Computer simulation results suggest, that the gel-fluid transition temperature can be lowered or raised by a proper selection of anionic-cationic amphiphilic modifiers. Moreover, the effect of ionic strength of the membrane medium and dielectric permeability of the hydrophobic part of membrane, where a shift of the dielectric constant from 5 to 80 occurred, has been studied. It was also shown that addition of alkyl molecules to the membrane, which are the counterions of alkyl modifier molecules added earlier, almost doubles the number of incorporated membrane modifiers. Membrane permeability analysis shows that membrane permeability, at temperatures lower than the gel-fluid transition temperature, should be more related to the number of small domains (cluster area) and less to the interfacial area.

Keywords: Monte Carlo simulation, membrane modifiers, phospholipids, Pink model, membrane, membrane simulation, gel-fluid transition

1. Introduction

The lipid phase of biological membranes seems to be essential for cellular function, because it can affect the activity of some membrane enzymes and a cell leakage. A number of biologically active compounds, especially amphiphilic, may modify the properties of membranes when incorporated into the lipid phase. This may result in a change in a local membrane permeability, leading to a disturbance in the physiological distribution of substances. The amphiphilic compounds incorporated into membranes may change their fluidity. Depending on type and concentration, these compounds may induce selective associations of the membrane lipids into domains which phase transition temperatures will differ significantly from that of unmodified membrane. Model studies concerning the ability of such compounds to be incorporated into membranes and to modify them, may prove helpful in effective application of these compounds in practice. Making use of the observed synergistic action, a desired effect may often be obtained with lower doses, and thus with lesser harmful effect on the environment. Some interesting suggestions may be deduced from a computer simulation. A literature study has shown that the Monte Carlo simulation, based on the ten state Pink's model, gives a good description of the gel-fluid transition and the influence of chain length of some amphiphilic compounds on membrane properties [1, 2].

2. The Model and Calculation Method

The effect of anionic-cationic mixtures on the lipid membrane properties was studied by a computer simulation method based on the Pink's model [3]. The effect of such compound mixtures with alkyl chains of various lengths (14, 16, 18 carbon atoms) on membranes formed of molecules with 16 and 18 carbon atoms alkyl chains has been analysed. According to the model each hydrocarbon chain can assume one of the ten possible configurations characterised by an internal energy E_a, the cross-section area of the acyl chain A_a, and an internal degeneracy D_a. For the fluid phase the most probable is the highly excited state with the largest internal energy and cross-section area. The opposite state, with the lowest values of the afore mentioned quantities, is the all-trans configuration.

The lipid membrane is modelled as the two mutually nonintereacting triangular lattices. Periodical conditions, imposed in both horizontal and vertical directions, make the model membrane a closed surface. The model assumes that each site (node) of a triangular lattice is occupied by a hydrocarbon chain which may exist in any of the ten states. According to this assumption one double-chain lipid molecule occupies two nodes of the lattice, and each chain is treated separately. Besides of model double-chain lipids, single-chain molecules with charged polar heads were considered. Using the screened Coulomb potential ψ in the form [4]

$$\psi = \frac{2Q\exp(-\kappa r)}{4\pi\varepsilon_o r}$$

where $\kappa^2 = \frac{2e^2 I N_A}{\varepsilon\varepsilon_o kT}$ and κ is the inverse Debye length, the pair charge Q_i, Q_j interaction energy U is given: $U = \frac{2Q_i Q_j \exp(-\kappa r)}{4\pi\varepsilon\varepsilon_o r}$, ε_o – permittivity of vacuum, ε – dielectric constant of the environment, kT – thermal energy, I – ionic strength, N_A – Avogadro's number, e – electron charge.

To examine the effects of electrostatic interactions of the charge modifiers the two cases were considered:

- the interaction between all charged molecules within lipid membrane or
- the interaction limited to the nearest neighbouring molecules.

The energy of interaction between an ion and rotating dipole (the polar head of lipid molecules) was neglected. However, the hydration shell of the polar part of the lipid bilayer was taken into account via the dielectric constant ε. The dielectric constant varied from 8 to 80. The model of aqueous phase contained monovalent ions mimicking the NaCl solution.

Thus, the Hamiltonian of the model considered is the following:

$$H=\sum_{i}\sum_{a}(E_a+\pi A_a)L_{ia}-\frac{j_o}{2}\sum_{i<>j}\sum_{a,b}\sqrt{C_aC_b}\,I_{ai}I_b(L_{ia}L_{jb}+L_{ja}L_{ib})+$$
$$\frac{\gamma}{2}\sum_{i<>j}\sum_{a,b}|d_a-d_b|(L_{ia}L_{jb}+L_{ja}L_{ib})+\frac{1}{2\pi\varepsilon_o\varepsilon}\sum_{i<>j}\frac{Q_iQ_j}{r_i+r_j}\exp(-\kappa(r_i+r_j))$$

where: L_{ia} is the occupation variable (0 or 1) of a state of the acyl-chain site i, J_0 is the coupling constant, E_a is the internal conformation energy, A_a is the cross-section area, I_a is the shape-dependent nematic parameter, π is the internal pressure, γ is the mismatch interaction constant, d_a is the acyl chain length, $Q_{i,j}$ is the electrical charge, $r_{i,j}$ is the effective radius, $C = 1/4\,\pi\,\varepsilon_0\varepsilon$.

The Metropolis method was used to study the lipid chain conformational changes and to obtain the thermodynamic averages. The studied lattice was composed of 10 000 nodes. The system was equilibrated for the 200 Monte Carlo steps per site in a given temperature, then 1000 steps per site were performed during which thermodynamic quantities were calculated [7]. Next the temperature was increased by 0.5 K and the procedure was repeated. The temperature was changed in the range 300-320 K and 320-335 K for the DPPC and DSPC model membrane, respectively.

3. Results and Discussion

When two model set-ups are compared, i.e. electrostatic interactions was calculated for the entire matrix or restricted to the interactions between nearest neighbours, the resulting shift in gel-fluid transition temperature was 0.5 K. That is why in further study only the nearest-neighbour electrostatic interaction was considered.

As mentioned earlier, the membrane medium constituted the solution of monovalent ions, which modelled NaCl solution. The increase of ionic strength by one order of magnitude (0.01–0.1M) causes the 1K increase in the gel-fluid phase transition temperature. As expected, this effect decreases with decreasing the dielectric constant of the medium.

Figures 1 and 2 show the dependence of the average cross-section area per chain on temperature for the membrane formed of the model molecules DPPC (Figure 1) and DSPC (Figure 2), modified with the anionic and cationic amphiphilic modifiers. At the gel-fluid transition temperature, a break in the cross-section area per chain occurs [2].

Calculations show that the shift of the gel-fluid transition temperature depends on the hydrocarbon chain length of modifier molecules.

The longer is the chain of anionic amphiphilic modifier molecules the stronger is the effect on the gel-fluid transition temperature, i.e. the transition temperature decreases with the chain length. The DPPC and DSPC model membranes modified

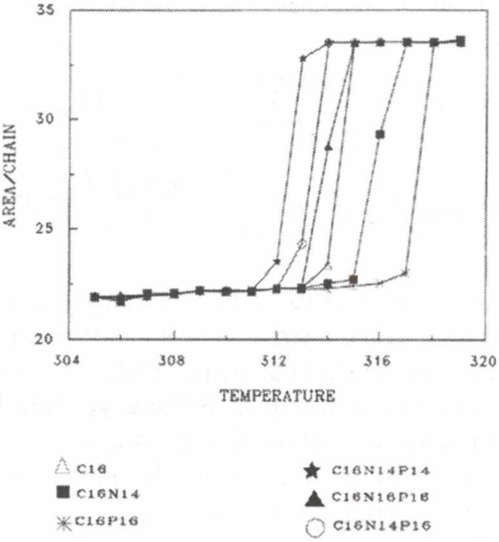

Figure 1. *Temperature dependence of the average cross-section area per chain for the modified membrane formed of the model DPPC molecules. Model DPPC membrane modified with the anionic modifier molecules with „a" carbon atoms in the alkyl chains is denoted by C16Na, membrane modified with cationic modifier with „b" carbon atoms in the alkyl chain — C16Pb. Consequently, C16NaPb describes the DPPC model membrane modified with a mixture of anionic and cationic modifiers.*

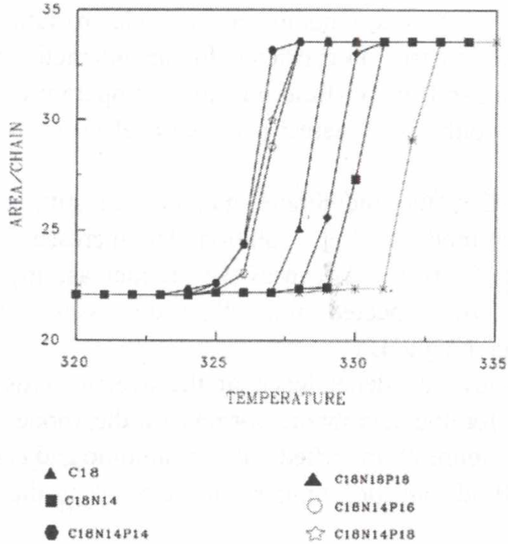

Figure 2. *Temperature dependence of the average cross-section area per chain for the modified membrane formed of the model DSPC molecules. Model DSPC membrane modified with the anionic modifier molecules with „a" carbon atoms in the alkyl chain is denoted by C18Na, membrane modified with cationic modifier with „b" carbon atoms in the alkyl chain - C18Pb. Consequently, C18NaPb describes DSPC model membrane modified with a mixture of anionic and cationic modifiers.*

with the mixture of negative and positive 14 carbon atoms chain modifiers, show the higher transition temperature when compared to the pure lipid membrane.

As shown earlier [5], modifying the membrane with the mixture of anionic-cationic amphiphilic compounds, which alkyl chains are of the same length as those of membrane lipids, practically does not change the gel-fluid transition temperature.

The membrane area may be divided into three fractional contributions, the fractional bulk area, the fractional cluster area, and the fractional interfacial area. In the minimal model [6, 7] it was assumed that the interface gives the major transmission rate.

Our studies of the membrane permeability of the interfacial area did not give any correlation between the permeability and modifier concentration. Therefore, we decided to concentrate on the second term responsible for the membrane permeability — i.e. the cluster area. We have examined different minimal sizes of clusters that might be responsible for the membrane permeability. The best results were obtained for the cut-off clusters size equal to four chains in the fluid conformation at 300 K (that is the temperature of earlier experimental studies [8, 9]).

Figures 3 and 4 show the changes in permeability of membranes built of the model DPPC (Figure 3) and DSPC (Figure 4) molecules, depending, on the type of modifier used, assuming, that the permeability is proportional to the number of domains (cluster area) built of at least 3 chains, in fluid state that surround a chain being also in fluid state. The permeability considered is referred to a permeability of unmodified membrane (relative permeability). The concentration of modifiers is always the same and equal to 4% of the membrane surface.

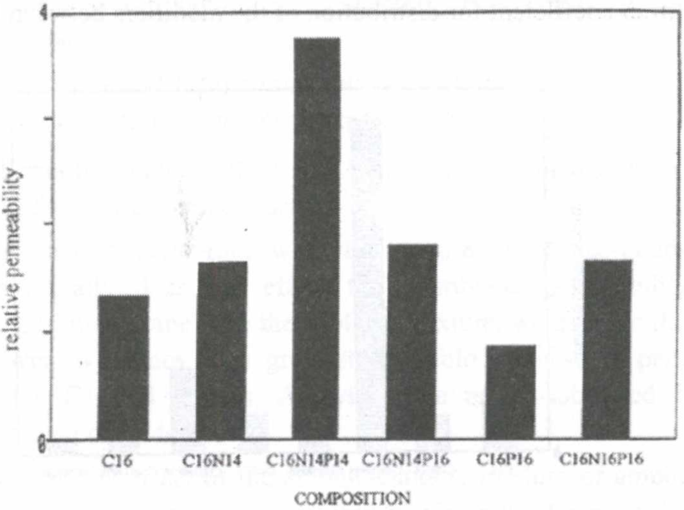

Figure 3. *Relative DPPC membrane permeability P/P_0 (relative to unmodified membrane) as a function of membrane composition. The amount of modifier molecules constitutes 4 percent of the total discrete membrane area. The lower cut-off in the cluster size was chosen to be three fluid chains surrounding a chain in the fluid state.*

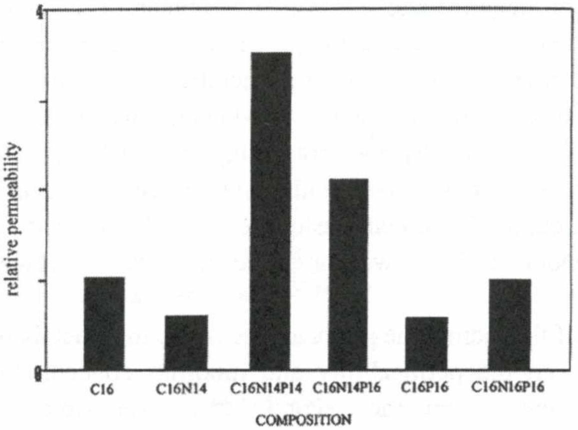

Figure 4. *Dependence between membrane composition and relative DSPC membrane permeability P/P_0 (relative to unmodified membrane). The ammount of modifier molecules constitutes 4 percent of the total discrete membrane area. The lower cut-off in the cluster size was chosen to be three fluid chains surrounding a chain on the fluid state.*

Figure 5 shows an analogous relationship for DPPC membrane, but the assumed permeability criterion is the number of domains formed of 6 chains in fluid conformation that surround a chain in fluid conformation. For the membrane formed of DSPC that criterion proved to be strong, because it rendered the membrane almost impermeable.

From those results it follows, that membrane permeability at the temperatures lower than the gel-fluid transition should be proportional to the number of small domains (cluster area). Introducing chemical potential to the model [10, 11], one can study a partition coefficient for distribution of the modifiers between the lipid phase

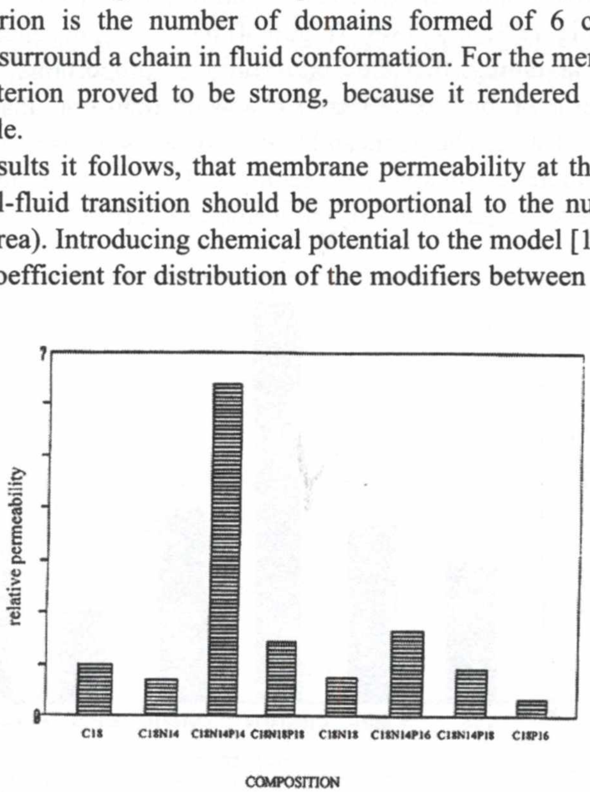

Figure 5. *Relative DSPC membrane permeability P/P_0 (relative to unmodified membrane) dependence on membrane composition. The ammount of modifier molecules constitutes 4 percent of the total discrete membrane area. The lower cut-off in the cluster size was chosen to be six fluid chains surrounding a chain on the fluid state.*

and medium. Figure 6 shows the relation between the number of incorporated modifiers, expressed as the surface percent, and magnitude of the chemical potential. As it is seen in the figure, the greater the difference in a chain length the smaller number of modifier molecules incorporated into the membrane.

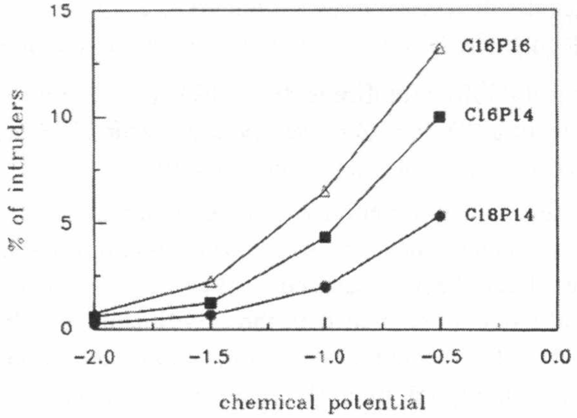

Figure 6. *Dependence of the number of intruder molecules on chemical potential μ.*
C16P16 - DPPC model membrane modified with cationic modifier with 16 carbon atoms alkyl chain,
C16P14 - DPPC model membrane modified with cationic modifier with 14 carbon atoms alkyl chain,
C18P14 - DSPC model membrane modified with modifier with 14 carbon atoms alkyl chain.

Comparing the plots of the mean surface area per chain versus temperature for the membranes formed of C16 and C18 (DPPC and DSPC membrane models) and modified with the N and P compounds of different chain lengths, one can draw the following conclusions:

- each of the N and P type compounds acting alone increases the gel-fluid phase transition; the more the longer the chains,
- the shorter the chains of the *P* and *N* molecules acting together the lower the gel-fluid transition temperature.

Modifying the C16 membrane with the mixture of anionic-cationic N16P16 compounds practically does not effect the membrane permeability. Whereas, modifying the C16 membrane with the N14P16 mixture, we observe the increase of permeability over two times. The greatest, five-fold increase in permeability, is observed for the C16P14 system. Analysing the results obtained for the C18 membrane, one can conclude that:

- the greater the effect of the anionic-cationic mixture of amphiphilic compounds on the membrane permeability the bigger the difference between chain lengths of the lipid and modifier molecules,
- the modifier molecules with the chains of the same lengths as those of the lipid molecules do not alter lipid membrane permeability.

Investigation of the partition coefficient of the modifier between the membrane and medium allowed to conclude:

- the amount of modifier molecules incorporated into the lipid membrane increases slightly as the temperature approaches the phase transition temperature,
- the greater the difference between the alkyl chain lengths of modifier and lipid molecules the less modifiers incorporate into the membrane,
- incorporating into membrane the alkyl modifier molecules, that are counterions of alkyl modifiers incorporated earlier, increases the number of earlier incorporated molecules almost two times.

The model presented may be applied to make qualitative interpretations of the observed membrane phenomena. The simplifying assumptions of the model and their consequences have been discussed earlier [12]. Nonetheless, the results obtained are in qualitative agreement with the experimental results obtained on the liposomes modified with the amphiphilic anionic-cationic compounds: N-dodecyloxymethylene-N-methylmorpholi-nium chloride and sodium alkylsulphonates [8, 9].

Acknowledgement

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References

[1] Ipsen J. H., Mouritsen O. G., Bloom M., *Relationships between lipid membrane area, hydrophobic thickness, and acyl-chain orientational order*, The effects of cholesterol. Biophys. J. **57**: 405-412 (1990)

[2] Mouritsen O. G., Boothroyd A., Harris R., Jan N., Lookman T., MacDonald L., Pink D. A., Zuckermann M. J., *Computer simulation of the main gel-fluid phase transition of lipid bilayers*, J. Chem. Phys. **79**: 2027-2041 (1983)

[3] Pink D. A., Green T. J., Chapman D., *Raman Scattering in Bilayers of Saturated Phosphatidylcholines*, Experiment and Theory. Biochemistry **19**: 349-356 (1980)

[4] Stankowski S., *Surface charging by large multivalent molecules. Extending the standard Gouy-Chapman treatment*, Biophys. J. **60**: 341-351 (1991)

[5] Kubica K., *The effect of amphiphilic counterions on the gel-fluid phase transition of the lipid bilayer*, Appl. Math. Comp. **87**: 261-270 (1997)

[6] Ipsen J., H., Jorgensen K., Mouritsen O. G., *Density fluctuations in saturated phospholipid bilayers increase as the acyl-chain length decreases*, Biophys. J. **58**: 1099-1107 (1990)

[7] Mouritsen O. G., Jorgensen K., Ipsen J. H., Zuckermann M. J., Cruzeiro-Hansson L., *Computer Simulation of Interfacial Fluctuation Phenomena*, Physica Scripta **T33**: 42-51 (1990)

[8] Kuczera J., Fogt A., Kubica K., Przestalski S., Witek S., Plucinski J., *Effect of a surface active anions on the activity of dedecyloxymethylene-methylmarpholinium chlorides as modifier of sulphate ion transport across lecithin liposome membrane*, Studia Biophysica **133**: 209-220 (1989)

[9] Sarapuk J., Kleszczynska H., Przestalski S., Kilian M., *Biological activity of N-dodecyloxymethylene-N-methylmorpholinium chloride enhanced by sodium sulphonates*, Tenside, Surfactants, Detergents **29**: 349-351 (1992)

[10] Jorgensen K., Ipsen J. H., Mouritsen O. G. Bennett D., Zuckerman M. J., *A general model for the interaction of foreign molecules with lipid membranes: drugs and anaesthetics*, BBA **1062**: 227-238 (1991)

[11] Jorgensen K., Ipsen J. H., Mouritsen O. G., Bennett D. and Zuckerman M. J., *The effects of density fluctuations on the partitioning of foreign molecules into lipid bilayers: Application to anaesthetics and insecticides*, BBA **1067**: 241-253 (1991)

[12] Kubica K., *Pink's model and lipid membranes*, Cellular and Molecular Biology Letters **2**: 257-263 (1997)