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ELECTRIC INTERACTIONS AT THE LIPID MEMBRANE SURFACE

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Abstract: This work presents the results of an experimental study and of computer simulations concerning electric interactions in the surface layer of egg yolk lecithin (EYL) liposome membranes. The surface layer is formed by EYL polar heads, which possess features of electric dipoles, and positive charged polar heads belonging to admixtures of quaternary ammonium salts (AS). The results of the experimental study are in good agreement with the ones of the computer simulations. It was found that fluidity of the membranes, at a given concentration of AS, obtains the extremal (minimal) value. Similarly, the binding energy of the dipoles-positive charges system behaves like that in computer simulations. Moreover, the locations of the fluidity extremum and those of the binding energy depend on the charge of the AS polar heads as well as on the degree of electric interactions screening by the environment. At a certain optimal value of the screening coefficient, the energy of the system is the lowest (the most negative) and together with the rise in AS charge, the minimum of the energy moves towards its higher concentrations.

Key Words: Lecithin Liposomes, Fluidity, Quaternary Ammonium Salts, EPR, Dipole Matrix, Binding Energy, Computer Simulations

INTRODUCTION

For many years, in the laboratories of Professor Witek (Institute of Polymer Technology of the Technical University of Wrocław), Professor Przystalski (Department of Physics and Biophysics, Agricultural University of Wrocław) and in EPR Department, Institute of Physics, Opole University, intensive research has been conducted concerning interactions between many quaternary ammonium salts (AS) and biological or model membranes. In biological tests, the compounds display bactericidal, algicidal and fungicidal properties [1], and by interacting with erythrocytes, they accelerate the hemolysis process [2].

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Model research has shown that AS introduced into the egg yolk lecithin (EYL) liposome membrane structure caused a growth in the speed of SO_4^{-2} ions permeability through these membranes [3] and also an increase in their fluidity [4]. The result of the AS interacting with the membranes depends on the structure of AS molecules (among others, on the CH_2 chain length and the geometrical dimensions of the polar head), their concentration, as well as on the electric charge of the polar head.

We focused our study on electrostatic interactions occurring in the surface layer of the membranes between positive charges of the polar heads of AS and those of EYL. From the physical point of view, EYL polar heads form electric dipoles localized in the liposome membrane-water solution interphase. The dipole properties follow from different locations of the geometric centers of the heads' charge; negatively charged phosphoric groups and positively charged choline ones. The electric interactions between the dipoles of EYL polar heads are strong and, as it may be supposed, they are responsible for the highest degree of ordering and the highest rigidity of this membrane area.

The aim of our work was to compare and test the correlation between the results of an experimental study (concerning fluidity of liposome membranes) and the results of computer simulations of the electric interactions in membrane surface layers.

RESULTS AND DISCUSSION

In our earlier studies conducted by means of the EPR spine probe method, concentrations of AS introduced into EYL liposomes were relatively high (10% and more in relation to EYL). At such concentrations, the presence of AS in the structure of the liposome membranes caused an increase in their fluidity (Fig. 1). The fluidity of the membranes was rising along with the rise in the concentration of AS. The results of the studies suggest that the electrostatic repulsion forces between positive charges of the polar heads of the AS molecules introduced into electrically neutral dipole environment were, among others, responsible for the rise in fluidity. Such an interpretation of the obtained results leads, simultaneously, to another conclusion, which – initially – was treated as a working hypothesis. The conclusion is the following: if the electric interactions in the surface layer have a considerable influence on the dynamic and structural properties of the membranes, then proportionally small AS concentrations should induce the opposite effect – rigidity of the liposome membranes structure (a decrease in fluidity). With a small number of the ion admixture dispersed in the environment of dipoles, the attraction forces between the ions and dipoles should be greater than the repulsion forces between the ions. Such a state can favor creation of aggregates in the shape of ions surrounded by dipoles, which – in consequence – may lead to stiffening of the membranes. However, along with the rise in the concentration of ions, the repulsion forces may become a

dominant factor which may cause a weakening of the structure of the membranes and a rise in their fluidity.

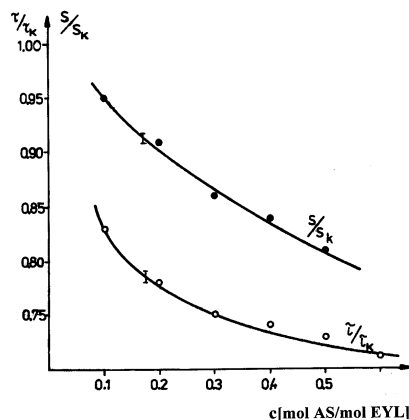


Fig. 1. Dependence of relative values of parameter τ (τ/τ_k) and S (S/S_k) of FA (1.14) and FA (10.3) spin probes, respectively, embedded into liposome membranes on the concentration (c) of AS with 12 carbon atoms in the alkyl chain. Spectroscopic parameter τ – quantity inversely proportional to the rotational speed of spin probe molecules, τ_k – parameter τ for a sample without AS. Parameter S – order parameter of spin probe molecules. The standard error for one series of measurements is given in the figure.

The experimental research carried out by means of the EPR spine probe method confirmed the hypothesis [5]. At concentrations of AS ranging from 2% to 5%, in relation to EYL, occurrence of minimal fluidity (maximal rigidity) of liposome membranes was observed (Figs 2, 3). The location of the rigidity maximum depends on the value of the AS polar head charge. For AS containing a head with a single charge - $(\text{CH}_3)_3\text{N}^+ \text{CH}_2 \text{COO C}_{10}\text{H}_{21} \text{Cl}^-$ (AS V-10) the concentration amounts to about 2%, while for the gemini type of AS V-10 - AS 2(V-10), with a double charge, it is shifted towards higher concentrations and stays in the range of values from 4% to 5%.

A similar result was obtained in other authors' studies. In [6] it was found that different surface-active amphiphilic compounds (including ionic ones) inhibit the hemolysis of erythrocytes at low concentrations, whereas at high concentrations they accelerate this process.

Fluidity is directly connected with the degree of freedom and mobility of the molecules which form membrane structure. Therefore, it is also combined with the forces responsible for their stability. The increase in fluidity caused by

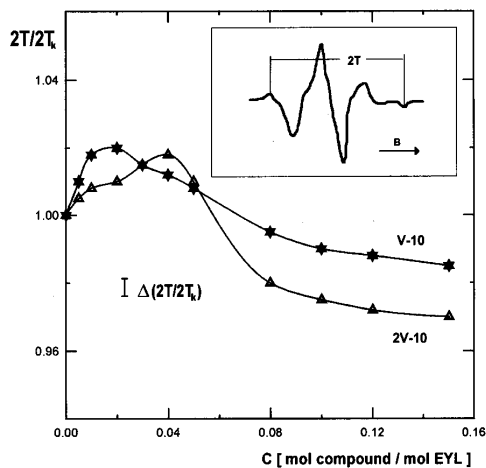


Fig. 2. Dependence of the relative value of parameter $2T$ ($2T/2T_k$) of FA (10.3) spin probe embedded into EYL liposome membranes on the concentration (c) of AS V-10 and AS 2V-10 admixtures. Parameter $2T$ – quantity proportional to the order parameter S . The figure presents the mean error of a measurement series and an EPR spectrum of FA (10.3) probe in the samples, on inset. The arrow in the inset means direction of magnetic field (B) increase.

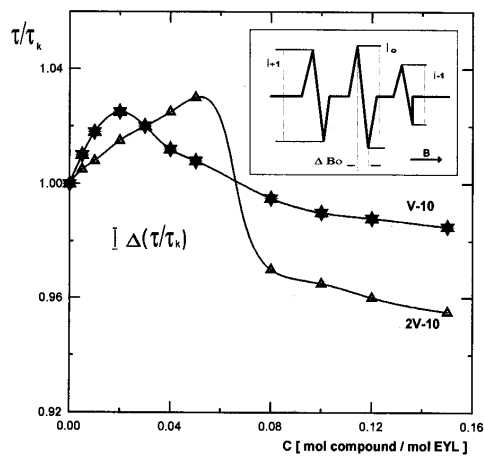


Fig. 3. Dependence of the relative value of parameter τ (τ/τ_k) of FA (1.14) spin probe embedded into EYL liposome membranes on the concentration (c) of AS V-10 AS 2V-10 admixtures. The figure presents the mean error of a measurement series and an EPR spectrum of FA (1.14) probe in the samples, on inset.

introduction of ion admixtures into the membrane surface layer is connected, as we can suppose, mostly with the weakness of the forces binding the lipid polar heads (at high admixture concentrations). On the other hand, the decrease in fluidity and the increase in binding forces is connected with low admixture concentrations.

Quantitative studies of relations between admixture concentrations and their charges on the one hand, and the interaction forces and binding energy of the layer components on the other hand, were conducted by means of the computer simulation method [7].

COMPUTER SIMULATIONS AND CONCLUSIONS

The computer model of the membrane has the shape of a flat, two-dimensional system, containing 121 electric dipoles which form an ordered matrix of rectangular or centered hexagonal symmetry. In this model the dipoles represent the polar heads of lecithin molecules. The system of dipoles was modified by admixtures of single (1Q) or double (2Q - in arbitrary units) point charges, which simulated the polar heads of AS molecules. The dipoles can not change their places in the matrix, but they rotate freely around the axes being perpendicular to its surface. At the beginning of the simulation process, the dipoles were set parallel, anti-parallel or at random in relation to one another (the arrangement was chosen by the generator of random numbers). The system defined in this way was subjected to simulation, whose effect was a gradual change in the orientation of the dipoles according to the resultant (instantaneous) moment of forces acting on each dipole and originating from all other dipoles and the admixed charges. For each dipole orientation the computer calculated the binding energy system (ϵ_s), which is the sum of the potential energies (ϵ) of its components (dipoles and ions).

In the mathematical procedures the interaction forces between electric charges were described by the Coulomb equation:

$$F = k q_1 q_2 / r^2 \quad (1)$$

where q_1 means the charge of a selected dipole or admixture ion, q_2 - charge of the other dipole or ion, r -- distance between q_1 and q_2 , k -- coefficient; the moment of force acting on the dipole by:

$$M = L * F \quad (2)$$

where L means the vector of dipole length, F - the vector of the resultant force acting on the dipole;

and the energy of interaction between the charges by:

$$\epsilon = - k q_1 q_2 / r \quad (3)$$

where $k q_1 / r = V_c$ means the Coulomb potential. (4)

After performing a series of steps, where each one included the state obtained in the one preceding it, the dipoles-admixture charges system attained the

equilibrium state, the minimal (the most negative) binding energy ($\epsilon_s = E$). Towards the end of the simulation process, the computer calculated energy E and relative energy E/E_0 (E_0 – minimal energy of the system devoid of admixtures), which is positive and in the equilibrium state has the maximal value.

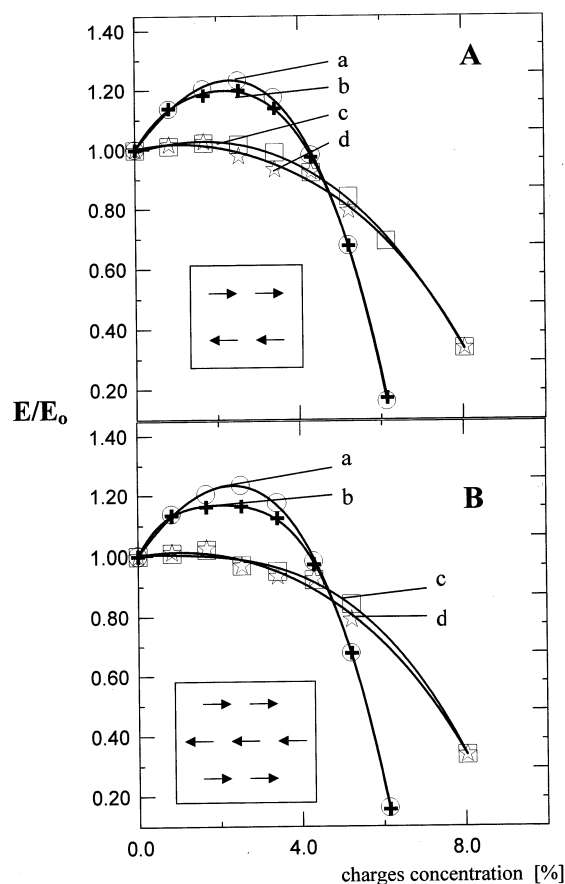


Fig. 4. Dependence of the relative value of the binding energy of the system in the equilibrium state (E/E_0) for rectangular (A) and hexagonal centered (B) geometry of the dipole distribution, on the ion admixture concentration. The E_0 symbol denotes the binding energy of the system without the ion admixture (in the equilibrium state), a-random dipole distribution at the beginning of the simulation process; charge admixture value equal to $2Q$ (in arbitrary units), b-anti-parallel dipole distribution at the beginning of the simulation process; charge admixture value = $2Q$, c-random dipole distribution, at the beginning of the simulation process; charge admixture value = $1Q$, d-anti-parallel dipole distribution, at the beginning of the simulation process; charge admixture value = $1Q$.

Tab. 1. Comparison of the relative values of the binding energy of the system (E/E_0) depending on the number and concentration of the admixture ions, for admixture charge equaling $2Q$ and $1Q$, respectively. At the start of the simulation the dipole system was set antiparallel or random in the rectangular (A) and centered hexagonal (B) matrix. For the matrix (A) $E_0 = -41.997$ (in arbitrary units) and for (B) $E_0 = -44.053$.

| | Number of ions | Ion concentration $c[\%]$ | Relative energy of the system (E/E_0) | | | |
|---|----------------|---------------------------|-------------------------------------------|--------|-------------------|--------|
| | | | Ionic charge $2Q$ | | Ionic charge $1Q$ | |
| | | | antiparallel | random | antiparallel | random |
| A | 0 | 0.00 | 1.000 | 1.000 | 1.000 | 1.000 |
| | 1 | 0.83 | 1.138 | 1.136 | 1.017 | 1.012 |
| | 2 | 1.68 | 1.182 | 1.204 | 1.027 | 1.023 |
| | 3 | 2.54 | 1.199 | 1.233 | 0.979 | 1.018 |
| | 4 | 3.42 | 1.138 | 1.174 | 0.933 | 0.991 |
| | 5 | 4.31 | 0.975 | 0.982 | 0.924 | 0.923 |
| | 6 | 5.22 | 0.676 | 0.677 | 0.793 | 0.842 |
| | 8 | 6.14 | 0.171 | 0.159 | - | 0.692 |
| | 9 | 8.04 | - | - | 0.337 | 0.337 |
| B | 0 | 0.00 | 1.000 | 1.000 | 1.000 | 1.000 |
| | 1 | 0.83 | 1.133 | 1.135 | 1.012 | 1.010 |
| | 2 | 1.68 | 1.161 | 1.203 | 1.025 | 1.021 |
| | 3 | 2.54 | 1.163 | 1.231 | 0.969 | 0.969 |
| | 4 | 3.42 | 1.123 | 1.172 | 0.934 | 0.950 |
| | 5 | 4.31 | 0.972 | 0.980 | 0.924 | 0.922 |
| | 6 | 5.22 | 0.676 | 0.676 | 0.790 | 0.841 |
| | 8 | 6.14 | 0.155 | 0.159 | 0.337 | 0.337 |

The results of the study (Tab. 1 and Fig. 4) displayed a qualitative agreement of computer simulations with the experiment. Dependence of the relative binding energy of the system (E/E_0) on the concentration (c) of $1Q$ charge admixtures obtains the maximal value at a concentration of about 1.7%. In the case of $2Q$ charge admixtures, the placement of the energy extremum is shifted towards higher concentrations, and the value of this extremum is greater than in the case of $1Q$ charge admixtures. As it may be expected, neither the kind of matrix formed by the dipole system, nor the arrangement direction of the dipoles at the beginning of the simulation process, have any considerable influence on the final state of the system's energy.

A next task which was undertaken in our study (conducted by means of the computer simulation method) concerned examining the influence of the

environment in which the dipoles-admixture charges system is placed, on electrostatic interactions between the system components. The presence of the environment lowers, as it may be expected, the forces of electrostatic interactions – it screens the forces. Due to this fact, the classical expression denoting the potential of Coulomb interactions (4), from which the binding energy was calculated, was replaced in the computer simulations by the expression of screened potential in the following form:

$$V_{scr} = (k q / r) \exp(-r/a) \quad [8].$$

The above expression includes a screening coefficient (a) (called “length of screening”), which represents the influence of the environment on electrostatic interactions between the charges. The length of dipole (L) was accepted in the study as the measurement unit of coefficient (a). A rise in the value of thus defined coefficient leads to a decrease in the influence of the environment on electrostatic interactions (the screening potential value increases). The increase in (a) in this model is equivalent to the increase in the distance between the interacting charges. If the value of the screening coefficient approaches infinity, then the screening disappears and the screening potential transforms into a classical Coulomb potential (non-screened):

$$\text{when } a \rightarrow \infty \quad \text{then } V_{scr} = V_c.$$

It follows from the study that together with the rise in the screening coefficient, the extremum of the binding energy of the dipoles-admixture charges system is shifted towards lower concentrations of the admixtures. Furthermore, the study proved that the highest, relative value of the binding energy (E/E_o) is obtained by the system in which the screening coefficient $a = 5L$. This value depends neither on the quantity of the charge of admixture introduced into the dipole system, nor on the kind of the matrix which the system forms. For the rectangular matrix, the concentration of the 1Q charge admixtures, corresponding to the maximal relative binding energy (E/E_o) of the system (at $a=5L$), amounts to about 2.5%, while in the case of 2Q charge admixtures – to about 4.3%. These concentrations are, approximately, equal to the concentrations of ions at which, in the experimental study, the EYL liposome membranes obtained the highest rigidity (the lowest fluidity) and – as it could be supposed – the highest relative binding energy.

Having compared the results of the experimental study with the ones of the computer simulations, it can be concluded that the maxima of the relative binding energy of the system dipoles of EYL polar heads - ions of AS polar heads have greater values in the case of admixtures containing polar heads of a higher electric charge. Moreover, the results of the study suggest that the system attains the greatest relative value of the binding energy at a certain optimal value of the screening coefficient of electrostatic interactions (in our study, $a_{optimal} = 5L$).

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