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The dynamics of the surface layer of lipid membranes doped by vanadium complex: computer modeling and EPR studies

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Abstract. Penetration of the liposome membranes doped with vanadium complex formed in the liquid-crystalline phase from egg yolk lecithin (EYL) by the TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) spin probes has been investigated. The penetration process was followed by 360 hours at 24°C, using the electron spin resonance (EPR) method. The spectroscopic parameter of the partition (*F*) of this probe indicated that a maximum rigidity of the membrane was at 3% concentration of the vanadium complex. Computer simulations showed that the increase in the rigidity of the membrane corresponds to the closure of gaps in the surface layer of the membrane, and indicates the essential role of the membrane surface in transport processes.

Key words: EPR probe • lipid membrane • membrane fluidity • Monte Carlo simulation

Introduction

The basic structure of a biological membrane is a lipid bilayer, which constitutes its core [1]. It serves as a foundation to which other elements determining individual properties of the membrane are attached. Knowledge about mechanisms affecting the properties of the lipid bilayer will allow for a better understanding of actual processes occurring in the biological membrane. Liposomes provide a good model for studying the physical properties of real biological membranes [2]. Both their molecular composition and geometric dimension can be precisely controlled, meaning that the studied objects are well defined. In order to better understand the processes in the lipid bilayer, we developed a computer model of the lipid membrane and conducted an EPR experiment. The comparison of the results collected during computer simulations, with the ones obtained by EPR measurement [3], showed that several properties of lipid membranes could be associated with the structure of their surface layer. The Coulomb interactions between polar heads of the surface layer seem to be essential for the membranes in the liquid state [4]. The explanation of the behavior of the Amphotericin B (AmB), put into lipid membranes [5], is an example of a successful application of that model. In this article the influence of a vanadium complex introduced into EYL the lipid membrane, and on the properties of the membrane, was investigated. Especially, the effect of time on the process of penetrating the membrane, by the TEMPO probe

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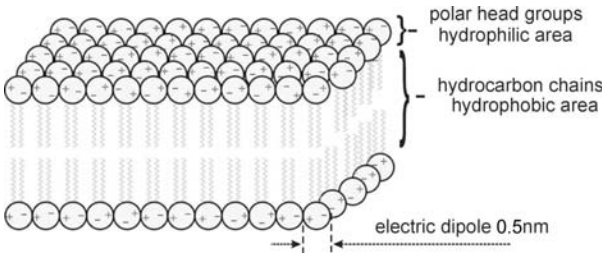

Fig. 1. The computer model of a lipid membrane.

and the influence of the vanadium complex in the process, was examined. EPR measurements and computer simulation were performed.

Materials and methods

Theoretical model

The theoretical model of the membrane surface considered here is an idealization of the surface of a real membrane with permanent dipole momentum. This model is oriented to the study of the properties of the dipole structure of a membrane surface. From a purely theoretical point of view, this is a two-dimensional crystal lattice built of electrical dipoles (Fig. 1), which are able to turn on their own axes perpendicular to the surface of the membrane as well as move over the plane of the membrane.

Apart from the mutual dipole-dipole electric interaction, they are influenced by the interaction of linked hydrocarbon chains. The chains strongly restrict the movement of the dipoles and prevent overlapping. As a result, a dipole behaves like a particle 'trapped' in a certain space by the linked chain, which acts as an 'anchor'. The strength of interaction between hydrocarbon chains has a big impact on the structure of the surface layer. Since we are interested in phenomena occurring in the surface layer, the interactions that occur within the membrane, we take into account in a phenomenological manner, using the Lennard–Jones potential. The Hamiltonian for the surface layer has the following form (1):

$$H = \sum_{(i)} \frac{p_i^2}{2m} + \sum_{(i)} \frac{L_i^2}{2I} + \sum_{(i<j)} U_{ij} + \sum_{(i<j)} V_{ij} \tag{1}$$

where L_i is the angular momentum of a dipole, m is the mass of the dipole, and I is its moment of inertia. U_{ij} (Eq. (2)) represents the energy of the dipole-dipole interaction between i-th and j-th dipole

$$U_{ij} = \frac{e^2}{4\pi\varepsilon_0\varepsilon_r}\left(\frac{1}{\left|\vec{d}_{ij}+\vec{a}_j-\vec{a}_i\right|}-\frac{1}{\left|\vec{d}_{ij}-\vec{a}_j-\vec{a}_i\right|}+\frac{1}{\left|\vec{d}_{ij}-\vec{a}_j+\vec{a}_i\right|}-\frac{1}{\left|\vec{d}_{ij}+\vec{a}_j+\vec{a}_i\right|}\right) \tag{2}$$

while V_{ij} (Eq. (3))

$$V_{ij} = 4\varepsilon\left[\left(\frac{\sigma}{\left|\vec{d}_{ij}\right|}\right)^{12}-\left(\frac{\sigma}{\left|\vec{d}_{ij}\right|}\right)^{6}\right] \tag{3}$$

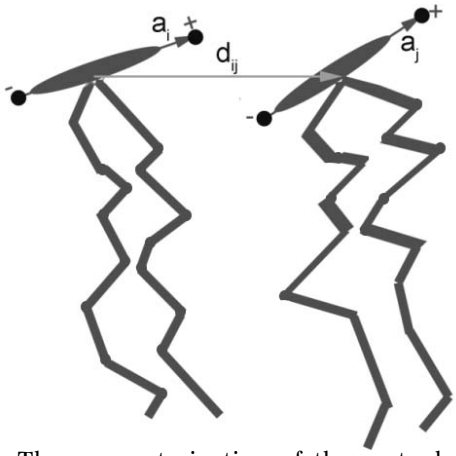

Fig. 2. The parameterization of the mutual dipoles orientation.

is the Lennard–Jones potential, which describes the interactions within the membrane. The meanings of particular vector parameters $\vec{d}_{ij}$, $\vec{a}_i$ are explained in Fig. 2. The dipole length $|\vec{a}_i|$ was set at = 0.5 nm, in accordance with the dimensions given in the work [6] for phosphatidylcholine.

The average distance between dipoles d_0 is known from literature [7] and allows us to determine the parameter σ in LJ potential (Eq. (4))

$$\sigma = 2^{-\frac{1}{6}} d_0 \tag{4}$$

Setting such value for σ ensures us that during the simulation the polar heads will be kept in proper distance one from another. The ε parameter is the depth of potential and could be seen as a phenomenological parameter of the rigidity of the internal part of the membrane. The bigger the value of this parameter, the greater the rigidity of the membrane. By changing the value of the parameter, we can get membrane in liquid crystal or gel form.

All the three degrees of freedom of a dipole are subject to change during the simulation. In our model, we have assumed that the impact of the environment is purely stochastic in nature. For this reason, the Monte Carlo method was applied, using the Metropolis' algorithm [8]. During the simulation, a Markov chain of states is generated. Every new state in the chain is obtained by random modification of the preceding one with the probability given by Boltzmann expression (5)

$$w = \exp\left(-\frac{\Delta E_p}{k_B T}\right) \tag{5}$$

where k_B is the Boltzmann constant, and T is the temperature. ΔE_p is the change of interaction energy during a simulation step. Every change in system state that leads to decreasing energy is accepted, whereas changes that increase the energy can be accepted or rejected, according to the probability given by Eq. (5). Starting from randomly generated initial state, we can observe a rapid decrease in the total interaction energy. After a certain time, the system reaches equilibrium state and the energy fluctuates around some average value and preserves some structural properties.

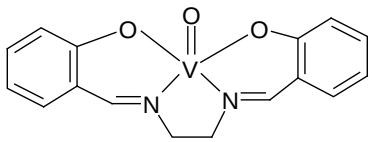

Fig. 3. The vanadium complex used in EPR measurement as an admixture.

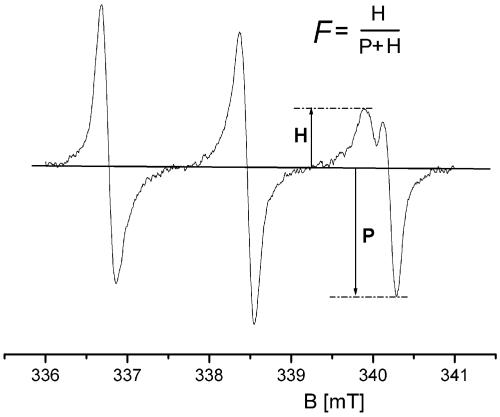

Fig. 4. EPR spectra of spin TEMPO probe placed in the membrane of liposome.

EPR experiment

The aim of the EPR experiment was to investigate the process of penetrating the membrane in a liquid-crystalline state, doped by the vanadium complex presented in Fig. 3, at different concentrations of the complex.

We expected that the complex will be mounted on the surface layer of the membrane and affect the penetration ability by the TEMPO probe. Liquid-crystalline liposomes (main phase transition temperature –5°C) were prepared from egg yolk lecithin (EYL). The liposomes were formed in distilled water in a sonication process by the means of an ultrasonic disintegrator (TECHPAN UD-20, Warsaw, Poland). EYL lecithin was extracted in the laboratory of the Faculty of Chemistry (Opole University). For a single sample of 2 ml by volume, the total effective sonication time was 8 min in alternate cycles including 30 s of sonication and 60 s of cooling. Concentration of the EYL in the particular samples was 0.04 M and that of the spin probe was 0.005% relative to the

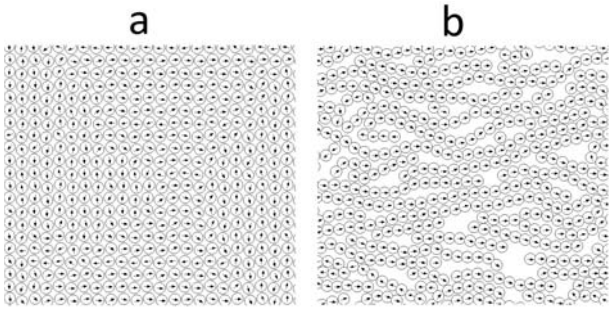

Fig. 5. Polar heads (circles) of the membrane surface and their orientations. The results of computer simulations for two different values of parameter ε: a – leading to a domain-like structure; b – leading to a liquid-crystalline phase.

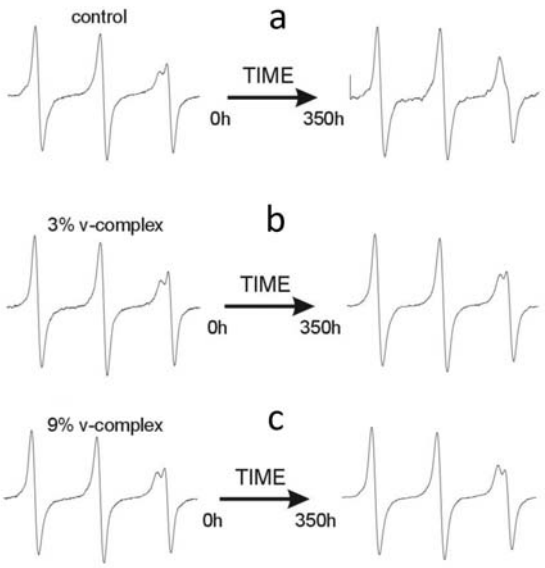

Fig. 6. The image of spectroscopic lines of the TEMPO probe recorded for the liposome membranes in the liquid-crystalline phase, (a) for the pure liposome, (b) the liposome with 3% of vanadium complex, (c) the liposome with 9% of vanadium complex, at the beginning of the measurements and after 350 h. The calculated values of the parameter. *F*: Control F_0 = 0.320 after 350 h F_{350} = 0.549, 3% V-Complex F_0 = 0.318 after 350 h F_{350} = 0.368, 9% V-Complex F_0 = 0.328 after 350 h F_{350} = 0.385.

lecithin. The 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) spin marker was added to the sample during the sonication process. Vanadium complexes were introduced into the samples before the liposomes were formed. The dopant concentration was 3 and 9% (molar) relative to the EYL quantity. Investigations were carried out at a constant temperature 24°C using the EPR spin probe technique, the spectra were collected from the moment the additive had been fed in and then for the following 350 h (Fig. 6). Between measurements the samples were stored in the dark at 24°C. From the spectrum of this spin probe, the spectroscopic parameter of partition (*F*) of this probe into the membrane and its environs was determined. The partition parameter *F* is defined as the ratio of the low-field line amplitude of the EPR spectrum of a spin probe placed in lipid medium (*H*) and the amplitude of the high-field line of a probe placed in aqueous medium (*P*) (Fig. 4). The value of *F* is connected, among others, with the fluidity of the membrane [9].

Results and discussion

Simulation results

The simulations were performed for different parameters of ε (Eq. (3)); depending on the value of parameter ε, we obtain the structure of the membrane surface that resembles the domain-like structure Fig. 5a, or the liquid-crystalline phase Fig. 5b.

The domain structures (Fig. 5a), typical for the gel phase, do not have evident gaps in the interphase. In contrast, the liquid-crystalline structures (Fig. 5b)

have a number of gaps that can be noted in the distribution of dipoles, which in real conditions, correspond to the defects in the surface layer of the membrane. These defects can be responsible for the penetration of the membrane by molecules. In order to verify this hypothesis, a series of the ESR experiments were carried out, which confirmed the existence of such penetration only in liquid crystal state [4]. Recent measurements have shown that also in the case of membrane in the liquid-crystalline state, it is possible to block the penetration channel of the membrane, by doping it with a vanadium complex. A detailed description of these measurements is presented in the results section.

EPR results

From Fig. 6a, we can read that in the case of the control sample (liposomes without the admixture), after $t = 350$ h from the beginning of the experiment, a significant proportion of the TEMPO probe, passed from the water to a lipid environment, which signifies a significant increase in the peak closer to the central line (and as a result, an increase in F parameter), compared to the state at $t = 0$ h.

However, quite the opposite effect can be observed for the sample with 3% concentration of the complex (Fig. 6b), here the parameter F decreased only slightly. Hence, we can conclude that in this case the spin probes have been displaced from the interior of the membrane. The concentration of 3% was not chosen by chance; from our earlier work, with different ionic admixtures, we know that for this value of concentration of admixtures a lipid membrane reaches the maximum of rigidity [10–12]. This similarity in obtained data suggests that also in the case of the vanadium complex we have to deal with increasing of the rigidity of the membrane. Such interpretation seems to be confirmed by the next measurement for 9% concentration of the complex (Fig. 6c), in which liposome membrane still maintains a low fluidity, but slightly higher than in the case of 3% concentration of admixtures.

As it was shown in earlier studies [4, 5], surface layer of a membrane in liquid state possesses a lot of defects, which enables easier penetration of the inner part of a lipid bilayer by TEMPO probe. Together with decreasing the membrane fluidity, polar head groups in the surface layer close the pores, which gradually disables the penetration of the interior part of the membrane by TEMPO probe. This phenomenon can be observed in Fig. 6. Such behavior of the membrane, corresponds well with the results presented in [10–12], and indicates a good correlation with computer simulations.

Conclusions

The EPR experiment and computer simulations have indicated that there is a correlation between the rigidity of the membrane and the structure of the surface layer composed of the polar heads. In the case of the dominant role of interactions between the hydrocarbon chains, the membrane is rigid and imposes its arrangement on the surface layer. The minimum energy of the interactions between the polar heads leads to a domain-like structure of the dipole orientations. If the interaction between the chains is weaker and the heads can move over the surface, then the interactions between the polar lipid heads tend to organize the surface layer to a liquid-crystalline like structure. This structure corresponds to the liquid-crystalline phase of the lipid membrane and allows easy penetration of the membrane by the TEMPO probe. However, the introduction of some ionic admixtures into the surface layer of the membrane leads, at concentration of about 3%, to stiffening of the membrane. This can be interpreted as a forced phase transition within the membrane, caused by increased tension in the surface layer. It seems to us that the experiment with vanadium complex as the admixture presented here could be interpreted in a similar way like the experiment with ionic admixtures [4].

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