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MEMBRANE DOMAIN ALTERATION UNDER THE ACTION OF BIOLOGICALLY ACTIVE SUBSTANCES: AN EPR STUDY

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Plasma membrane is an active barrier with heterogeneous distribution of lipids and proteins arranged in several coexisting domains with different fluidity characteristics. Fluidity of the whole plasma membrane reflects the ordering and dynamics of phospholipid acyl chains in specific membrane domains, as well as the fraction of each domain in the membrane. Different biologically active substances can strongly influence the fluidity characteristics, in this way they affect processes in the membrane such as transport, enzyme activities and expression of the receptors and consequently cell growth, differentiation and transformation. In this paper the electron paramagnetic resonance method (EPR) is described by which it is possible to characterise the domain structure in the membranes of biological samples. The method is based on the computer simulation of the EPR spectra line-shapes of the membrane dissolved spin probes. In the model we take into account that the membrane is heterogeneous with several coexisting domains. The parameters, which describe ordering, dynamics and polarity of the spin probe environment in each domain as well as the proportion of individual domain in the membrane can be determined by the evolutionary optimisation of the simulated spectra to the experimental spectrum. This procedure allows the extraction of small changes in the membrane caused by different influences from the environment. The contribution of the relative portion of each domain can be distinguished from the contribution of fluidity alterations in the domain. Two examples are discussed: (i) The influence of cholesterol on the membrane fluidity alterations in alkyl-phospholipid liposomes; (ii) the difference in the domain structure of neutrophil membranes from blood (dormant neutrophils) and bronchoalveolar fluid in asthmatic horses (active neutrophils).

INTRODUCTION

Plasma membrane is highly selective filter device for active transport and signal transduction. It controls the entry of nutrients into the cells and exit of waste products out of the cells, generates difference in ion concentration between interior and exterior, acts as a sensor for exterior signals, allowing the cell to change in response to the environmental conditions (Alberts, Bray, Lewis, Raff, Roberts & Watson, 1989).

Membrane is a heterogeneous structure composed of lipids (phospholipids, cholesterol, sphingomyelin) and proteins (enzymes, receptors, transport proteins) with or without the attached oligosaccharides. Lipids in the membrane are arranged in the bilayer structure with polar parts oriented toward the water solution interface. Lipid bilayer is around 5 nm thick and can be treated as an anisotropic two-dimensional fluid in which constituent molecules undergo translational and rotational motion at a rate characteristic for viscous oil (Bloom, 1992).

Due to the heterogeneous composition the distribution of membrane constituents inside the lipid bilayer is not uniform. Motional freedom of phospholipids with long and highly saturated acyl chains is highly restricted; the molecules are in the gel phase at room temperature. On the other side the lipids with short chains and unsaturated bonds are in liquid crystal phase at room temperature, their ordering of the acyl chains is lower and lateral diffusion within the bilayer is faster. The phospholipids with similar physical characteristics tend to assemble together, as a consequence the phase separation in the membrane can occur, and several coexisting domains are formed with different motional freedom (Jørgensen & Mouritsen, 1995). Translational and rotational motional freedom of molecules inside the certain region in the membrane is characterized by membrane fluidity, which is inversely proportional to the membrane microviscosity.

Additionall heterogeneity in the membrane structure arises from the specific interactions between the membrane components e.g. protein-lipid interaction (Marsh, 1995) or due to the interaction between phospholipids and cholesterol (Simons & Ikonen, 2000). Cholesterol composes 30 % to 50 mol% of all lipids in eucaryiots. In this concentration range cholesterol induces formation of a new type of phase, that is more ordered than the liquid crystal phase of pure phospholipid system (liquid-ordered phase). In this way cholesterol tends to regulate or completely eliminates the

possible phase transitions of phospholipids from gel to liquid crystal phase. Besides, it decreases permeability for water molecules and enhances mechanical stability of bilayer (Bloom, 1992). Cholesterol prefferentially associate with highly saturated lipids, typically sphimgomielin creating a separate highly ordered phase, frequently called rafts (Simons & Ikonen, 2000), which is characterized by slow lateral diffusion and more restricted acyl chain motion in the bilayer. It is also dispersed in the liquid disordered phase constituting the more loosely packed fluid matrix of the membrane. Cholesterol is therefore an important membrane constituent that induce and maintain the membrane lateral heterogeneity.

The lipid phases determine the boundaries for lateral diffusion of proteins in each phases (Simons & Ikonen, 2000). By modulating lipid concentrations and external conditions, the dimension of the domains can be influenced and consequently also the diffusion of proteins in the membrane changes. This may play an important role in regulating membrane properties both under physiological conditions and in the pathogenesis of diseases. Therefore the knowledge about the domain structure and dimension of particular domains in the membrane is essential for understanding the complex mechanisms, which are regulated by membrane properties.

One of the methods by which it is possible to follow the physical characteristics of the membrane and to get some data about the domain structure of the membrane is electron paramagnetic resonance method with spin labeling (Ge & Freed, 1999; Žuvić-Butorac, Müller, Pomorski, Libera, Herrmann & Schara, 1999; Šentjurc, Zorec, Čemažar, Auersperg & Serša, 1998). By the conventional analysis of the EPR spectra, from some characteristic maxima and minima in the spectrum (Marsh, 1981; Curtain & Gordon, 1984), or by spectral subtraction (Mahaney, Kleinschmidt, Marsh & Thomas, 1992) an average order parameter and an empirical correlation time can be estimated, which give information about an overall membrane fluidity and fluidity changes caused by different external influences. In some cases by spectral subtraction the information about the distribution of spin probe between the less and more ordered membrane domains can be also evaluated. However, by computer simulation of the EPR spectral line-shapes, taking into account heterogeneity of biological membranes, more information about membrane domains can be obtained. In this work we would like to describe the method and possible types of information that can be extracted from the EPR spectral line-shapes simulation by evolutionary optimization of the spectral parameters.

ELECTRON PARAMAGNETIC RESONANCE

Electron paramagnetic resonance is spectroscopic method by which the paramagnetic substances, i.e. the molecules with non-paired electrons can be detected. For the investigation of membrane characteristics a lipophilic spin probes should be used, which incorporate primarily in the membrane bilayer of cells and/or tissues. The line-shape of the EPR spectra of such spin probe reflects the physical characteristics of its surrounding, and in this way gives the information about the number of different types of membrane domains, their fluidity characteristics as well as of the proportion of the domains in the membrane. In EPR experiment the term fluidity is used to describe the motional freedom of the spin probe within the membrane domains. It is described by order parameter (S), averaged deviation of the acyl chains from the normal to the bilayer plane ($S = 1$ for perfectly ordered crystals and 0 for isotropic fluid), and rotational correlation time (τ_c), time required for the molecules to forget what were their previous spatial orientations (Cader, Butterfield, Watkins, Hong Chung & Hennig, 1995) and describes the rate of rotational motion of the spin probe nitroxide group within the domain. From the EPR point of view domains represents the group of molecules with similar ordering and dynamics irrespective to its location in the membrane. More small regions with the same physical characteristics could not be distinguished from few large areas. Proportion of certain domain in the membrane as determined by EPR (d) depends on the partition of the spin probe between the domains. If the distribution of spin probe between the domains is not uniform the values obtained for the proportions will not correspond to the surfaces of domains. The mentioned parameters S, τ_c and d, together with the other, which will be defined latter, can be determined by the computer simulation of the EPR spectra lineshape, according to the best fit to the experimental spectrum.

EPR SPECTRA SIMULATION

To simulate EPR spectra the restricted fast-motion approximation procedure was used. For spin labeled cell suspension or tissue we take into account superimposed EPR spectra consisting of several spectral components, which correspond to

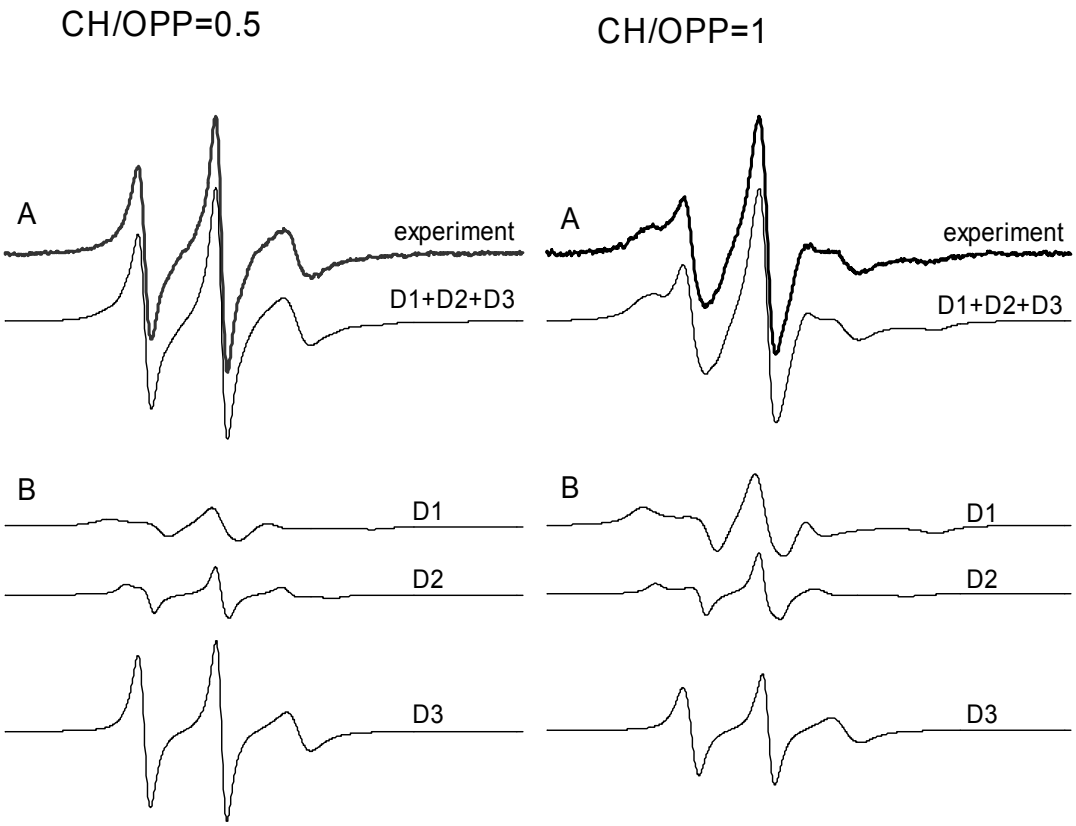

Fig.1 Typical EPR spectrum of MeFASL(10,3) in the membrane of OPP liposomes with CH/OPP = 0.5 mol/mol (left side) and CH/OPP = 1.2 mol/mol at 30 °C . **A. Thick line:** experimental spectrum; **thin line:** the best fit to the experimental spectrum, taking into account that the spectrum is the superimposition of the spectra of three coexisting domains (D1, D2, D3), as presented in Fig. 1B. **B.** Computer simulation of the EPR spectra (presented in Fig. 1A) of the three coexisting domains D1, D2 and D3. The corresponding parameters are presented in Table 1.

the coexisting membrane domains. They arise from various compartments (solution, rafts, outer and inner layer, aggregates) with different physical characteristics.

In the restricted fast-motion approximation the calculation of every spectral component involves three calculation steps (Štrancar, Šentjurc & Schara, 2000).

In the first step the magnetic tensors are averaged over fast stochastic restricted rotational motions of the spin probes to calculate the powder-like resonant field distribution. This is done according to the order parameter S for the assumed averaged orientation of the nitroxide group relative to the membrane normal vector. Since neighboring electric fields influence the electron density distribution the magnetic tensors (g and A) change with the environment of the spin probe nitroxide group. Therefore the polarity correction factors p_g and p_A are introduced, which act on the trace of the tensor and characterize the polarity of the individual component.

In the second step the EPR spectral line-shape is calculated in the motional narrowing approximation. The rotational dynamics within a particular domain is described with one effective rotational correlation time τ_c. Additional broadening (W) of the EPR spectral absorption lines due to the unresolved hydrogen superhyperfine splitting, dipolar interaction between the spin probe molecules and oxygen, together with some paramagnetic impurities (oxygen), external field inhomogeneity, modulation effects etc is also taken into account.

In the third step the convolution of the resonant field distribution with the first derivative of the line-shape is calculated for all spectral lines of each spectral component, which belong to the domains in the membrane.

Finally, all spectral components are summed with the corresponding weighting factor d, which takes into account the proportions of the spin probes in the particular domain in the membrane.

In summary, spectral simulation requires the following parameters for each spectral domain:

Table 1: Membrane characteristics for three membrane domains (D1, D2, D3) in CH poor (CH/OPP = 0.5 mol/mol) and CH- rich liposomes (CH/OPP = 1 mol/mol) at 30°C and 50°C. They are determined from the best fits of the computer simulation of the EPR spectra line-shape of MeFASL(10,3) spin probe to the experimental ones presented in Fig.1. The errors are estimated through covariance matrix analysis.

	CH/OPP=0.5 at 30°C			***CH/OPP=0.5 at 50°C***		
Domain	D1	D2	D3	D1	D2	D3
S	0.55 ± 0.01	0.26 ± 0.01	0.06 ± 0.01	0.40 ± 0.02	0.27 ± 0.01	0.05 ± 0.01
τ_c [ns]	0.5 ± 0.2	0.5 ± 0.2	1.40 ± 0.06	1.1 ± 0.4	0.4 ± 0.1	0.70 ± 0.01
w	2.5 ± 0.3	1.0 ± 0.2	0.60 ± 0.04	1.8 ± 0.4	0.2 ± 0.1	0.60 ± 0.01
p_a	1.01 ± 0.01	1.0 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.0 ± 0.01
d	0.38 ± 0.03	0.10 ± 0.03	0.50 ± 0.03	0.20 ± 0.02	0.00 ± 0.01	0.70 ± 0.02

	CH/OPP=1 at 30°C			***CH/OPP=1 at 50°C***		
Domain	D1	D2	D3	D1	D2	D3
S	0.68 ± 0.01	0.46 ± 0.02	0.12 ± 0.01	0.50 ± 0.01	0.31 ± 0.01	0.05 ± 0.01
τ_c [ns]	0.1 ± 0.1	1.3 ± 0.3	1.3 ± 0.1	0.1 ± 0.1	1.0 ± 0.2	0.80 ± 0.02
w	2.9 ± 0.2	0.4 ± 0.3	0.8 ± 0.08	3.0 ± 0.1	0.4 ± 0.1	0.70 ± 0.02
p_a	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	0.90 ± 0.01	1.00 ± 0.01
d	0.63 ± 0.04	0.20 ± 0.03	0.2 ± 0.03	0.50 ± 0.01	0.20 ± 0.01	0.40 ± 0.01

S: Order parameter, τ_c: rotational correlation time, p_a: polarity correction factor of hyperfine splitting constant and d: relative portion of a domain D in the membrane.

order parameter *S*, rotational correlation time τ_c, broadening constant *W*, polarity correction factors p_A and p_g, and the weighting factor *d*

OPTIMIZATION PROCEDURES

To navigate the optimization of the spectral parameters an objective function is introduced as a measure of the goodness of the fit of the simulated spectrum to the experimental one. The measure is the reduced χ^2 defined as

$$\chi^2 = \frac{1}{N}\sum_{i=1}^{N}\frac{\left(y_i^{exp} - y_i^{sim}\right)^2}{\sigma^2},$$

where y_i^{exp} and y_i^{sim} are experimental and simulated spectra intensities, respectively, σ is the standard deviation of the experimental points, and *N* is the number of points in the spectrum.

Optimization method used in this study is based on two basic optimization schemes. The deterministic and single-point optimization method (Simplex downhill) (Štrancar, Šentjurc & Schara, 2000) provides good results only if starting points are close to the solutions. This robust method converges relatively fast. However, it often leads to the convergence into a local rather than in global minimum. Partial but time-consuming solution to this problem involves the navigation of an experienced spectroscopist.

To introduce a method, which could eliminate the involvement of the spectroscopist, we use the stochastic and population-based genetic algorithm, which requires no special starting points and no user intervention. It is good at finding promising regions in complex search space but may have difficulties in fine-tuning. Therefore we coupled both methods into a hybrid evolutionary optimization method, which basically includes a genetic algorithm hybridized with local search (Simplex downhill), and some knowledge based operators (Filipič & Štrancar, 2001).

The results are at the level of the best results known so far for the one and two-domain problems and significantly better for the three domain problems. The errors are estimated through the covariance matrix analysis.

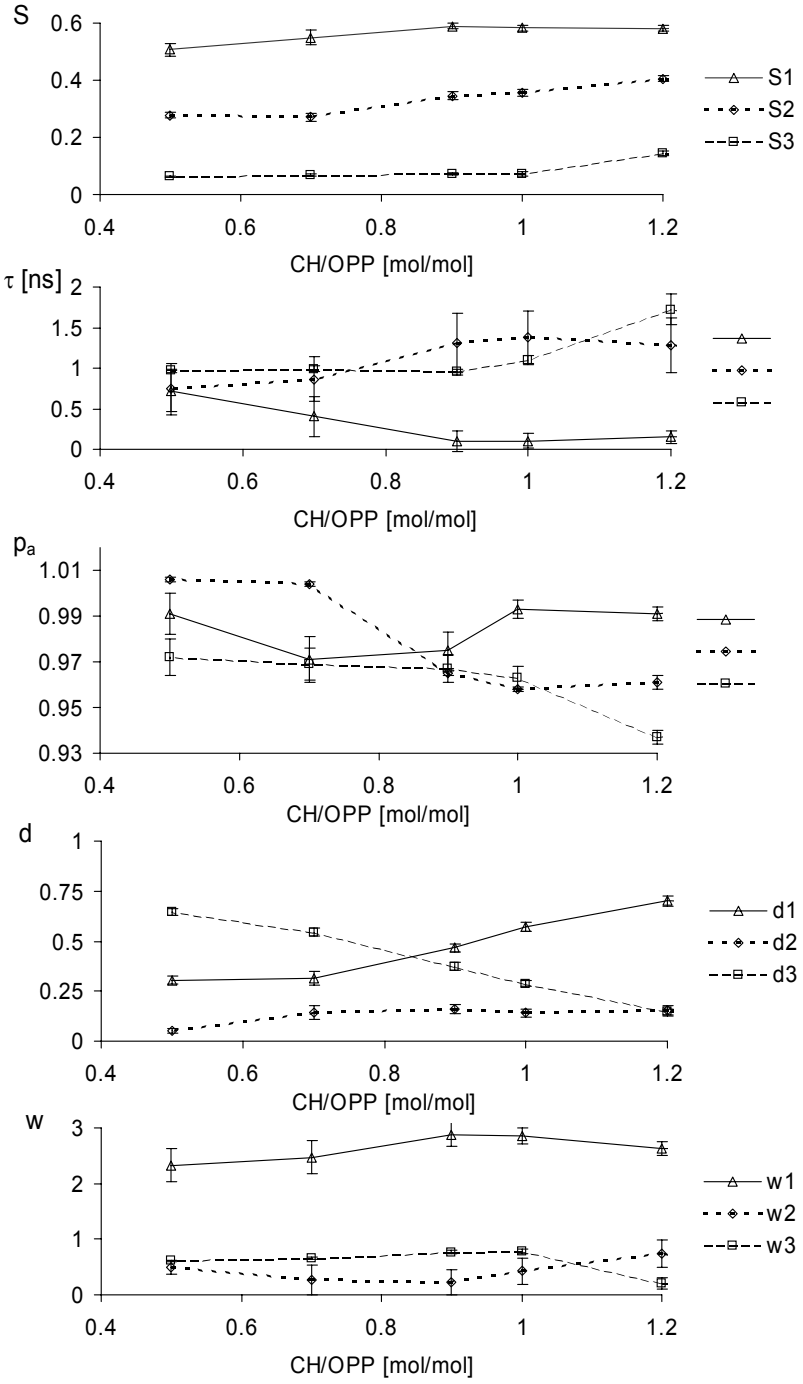

Fig.2. Influence of cholesterol concentration on the EPR parameters of MeFASL(10,3) in OPP liposomes at 40°C. ***S*** = order parameter, τ = correlation time, p_A = polarity correction of hyperfine splitting constant, ***d*** = relative portion of the domains and ***W*** = line width of the EPR spectra

EXAMPLES

Influence of cholesterol on membrane characteristics of alkylphospholipid liposomes.

Alkylphospholipids are physiologically active derivatives of lipids efficient in therapy of breast cancer. Till now the strongest antitumor effect was obtained for octadecyl-(1,1-dimethyl-4-piperidino-4-yl)-phosphate (OPP). This is amphiphilic substance, which forms micelles in the solution. The serious disadvantage of this substance is that it is cytotoxic at higher concentrations and produces haemolysis. It was shown recently that haemolysis could be prevented by liposomal formulation of OPP. The highest antitumor activity with less side effects can be obtained by preparing the liposomes in the form of stearically stabilised, negatively charged liposome suspensions with a molar ratio between cholesterol and OPP ratio lower than 1 (Zeisig, Arndt, Stahn & Fichtner, 1998). It was further found that the final liposomal formulation was accompanied with a formation of

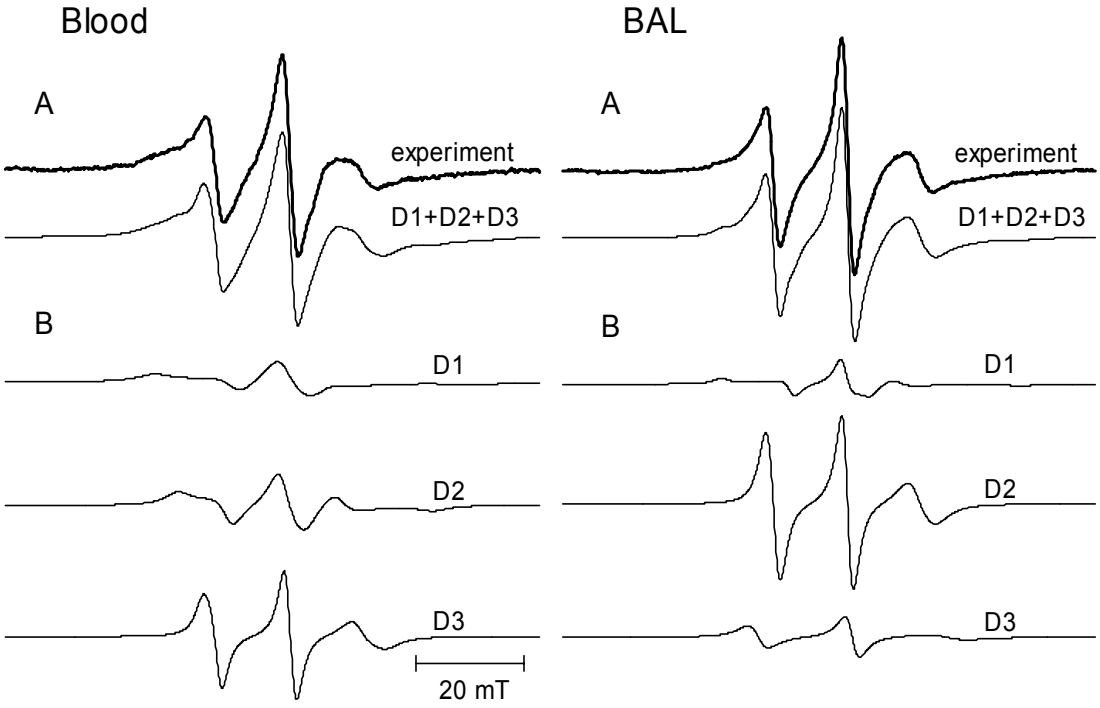

Fig.3 EPR spectra of MeFASL(10,3) in the membranes of neutrophils from blood (dormant neutrophils) and from bronchoalveolar fluid (BAL) (active neutrophils) obtained from horses suffering from COPD. A. Thick line: experimental spectrum; thin line: the best fit to the experimental spectrum, taking into account that the spectrum is the superimposition of the spectra of three coexisting domains (D1, D2, D3), as presented in Fig. 1B. B. Spectral components by which the best fits to the experimental spectra are obtained. The corresponding parameters are presented in Table 2.

OPP-micelles, which depend on the ratio between cholesterol and OPP (Zeisig, Müller, Maurer, Arndt & Fahr, 2001). This indicates that the liposomal composition, especially the amount of cholesterol in the membrane, influences not only the physical properties of the formulation, but also the resulting effects in biological systems. For better understanding of micelle formation as well as the interaction of OPP liposomes with cells in our previous study the influence of cholesterol, temperature and sterical stabilization of liposomal formulation on the membrane domain structure of liposomes was investigated. This may allow the optimal liposomal formulation to be selected for therapeutic use (Koklič, Šentjurc & Zeisig, submitted).

Here we would like to demonstrate the benefits of our approach where the membrane heterogeneity is taken into account in comparison to conventional analysis of the EPR spectra, where the membrane is treated as a homogeneous structure (Curtain & Gordon, 1984).

Liposomes used in this study consist of OPP, cholesterol, and negatively charged component, dicetylphosphate (2 mol%). The molar ratio of cholesterol to OPP (CH/OPP) varied from 15/10 to 5/10 (mol/mol). Multilamellar liposomes (MLV) with an OPP concentration of 10 mM were prepared by the lipid film/hydration method (Zeizig *et al.*, 1998). For EPR measurements liposomes were spin labelled with the lipophilic spin probe MeFASL(10,3) (5-doxyl of palmitic acid methyl ester) in a molar ratio of MeFASL(10,3) to all other liposomal components of 1/600 (Vrhovnik, Kristl, Šentjurc & Šmid-Korbar, 1998).

In Fig. 1 the EPR spectra of OPP liposomes labeled with MeFASL(10,3) at two different concentration of cholesterol are presented, together with the best fit of the calculated spectrum obtained as a superimposition of three spectral components (Fig. 1B). The EPR parameters by which the best fits are obtained are shown in Table 1.
From Fig. 1 one can see that the line-shape of the EPR spectra is very sensitive to the cholesterol concentration. By the conventional EPR analysis (Curtain & Grodon, 1984) from Fig 1 we can estimate the average order parameter as well as the empirical correlation time, which both increase after addition of cholesterol. Therefore we can conclude that with increasing concentration of cholesterol the membrane becomes more rigid. However, by the computer simulation of the EPR

Table 2: Membrane characteristics for three membrane domains (D1, D2, D3) of neutrophils isolated from blood (dormant neutrophils) or bronchoalveolar fluid (BAL)(active neutrophils) of a hors suffering from asthmatic disease. They are determined from the best fits of the computer simulation of the EPR spectra line-shape of MeFASL(10,3) spin probe to the experimental ones measured at room temperature (fig.3). The errors are estimated through covariance matrix analysis.

	Blood			***BAL***		
Domain	D1	D2	D3	D1	D2	D3
S	0.72 ± 0.03	0.51 ± 0.02	0.16 ± 0.01	0.60 ± 0.01	0.11 ± 0.01	0.03 ± 0.01
τ_c [ns]	0.8 ± 0.4	0.3 ± 0.2	1.10 ± 0.09	0.7 ± 0.2	1.30 ± 0.04	2.2 ± 0.3
w	3.0 ± 0.4	2.6 ± 0.2	0.80 ± 0.07	1.1 ± 0.2	0.80 ± 0.03	0.3 ± 0.1
p_a	1.10 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	0.90 ± 0.01	1.20 ± 0.01
d	0.40 ± 0.06	0.30 ± 0.06	0.20 ± 0.01	0.20 ± 0.04	0.50 ± 0.02	0.20 ± 0.03

S: Order parameter, τ_c: rotational correlation time, p_a: polarity correction factor of hyperfine splitting constant and d: relative portion of a domain D in the membrane.

spectra we can study membrane heterogeneity and characterize the membrane domains in more details. Spectra presented in Fig. 1 can be fitted as a superimposition of three spectral components. This means that in the liposome membrane there are at least three different types of domains with different physical characteristics. (In the figures and in tables they are denoted as D1, D2 and D3, starting with the most ordered domain).

From the parameters in Table 1 we can see that with increasing concentration of cholesterol not only the order parameter of the domains increases, but also the proportion of the domain with higher order parameter (D1) increases. It was already observed previously, that cholesterol is not distributed in the membrane uniformly (McMullen, Lewis & McElhaney, 1993). Various cholesterol-poor and cholesterol-rich regions are formed. With increasing cholesterol content the proportion of cholesterol-rich regions increases. At molar ratios of cholesterol against OPP (CH/OPP) close to 1 or more there are no regions in the membrane without cholesterol. This agrees also with our observation that the order parameter of the less ordered domain (D3 in Tab. 1) increases the most in this molar ratio range.

However, from the parameters presented in Tab. 1 we can get more information about the characteristics of the domains. The rotational correlation time τ_c is shorter in the domain D1 with the highest order parameter (S_1) and decreases significantly at higher concentrations of cholesterol. This indicates that the rotational motion of the spin probe alkyl chains is faster in the highly ordered domains. On the contrary, in the less ordered domains (D2 and D3) the rotational correlation time τ_c increases with increased cholesterol content (Fig. 2).

Very important parameter seems to be polarity correction factor p_A, which gives some information about the accessibility of water molecules to the spin probe. Interesting is its correlation with the order parameter S in the sense that the polarity correction factor in the most ordered domains is greater than in the less ordered domains (Fig. 2 and Tab. 1). From this correlation we can speculate that the disordered domains are less accessible to water molecules than the ordered domains, or that the spin probe in more ordered domains exchange faster between the water and lipid interface than in the less ordered domains. This is in accordance with the molecular dynamic simulation of the bilayer performed by Heller, Schaefer and Schulten (1993), which has shown that water molecules could penetrate deeper into the highly ordered regions in the bilayer and not so easily into the regions with low order.

The broadening parameter W seems to give some information about the distribution of the spin probe in the domain. Basically, the parameter W originates in spin-spin interaction as well as in some other experimental reasons. Broadening due to the hydrogen superhyperfine splitting, external field inhomogeneity, modulation effects etc, should be the same for all the domains. On the other hand, broadening due to the paramagnetic impurities, i.e. the amount of oxygen in the domain and local concentration of spin probe can vary between the domains. According to our recent results the broadening parameter W often becomes larger in the domains with high order parameter and lower in the less ordered domains.

This could be in favor of higher local concentration in the ordered domain, due to higher partition coefficient, or higher dipolar broadening due to more anisotropic environment, or both.

If we compare the data at low and high temperature (Tab. 1), we can see that the order parameter and rotational correlation time decreases with temperature. Additionally, some reorganization within the membrane occurs, which increases the proportion of less ordered domains.

Comparison of the domain structure of the membranes of neutrophils isolated from blood or bronchoalveolar fluid (BAL) of a hors suffering on asthmatic disease

Accumulation of neutrophils in lung is a prominent feature of chronic obstructive pulmonary disease (COPD) (asthma like syndrome). The activation of these cells, which results in the production of proteases and oxygen–derived free radicals, is thought to be important in the pathogenesis of the disease. The aim of this study was to explore the possibilities given by the computer simulation of the EPR spectra line-shapes to determine the changes in the membranes of neutrophils from blood (dormant neutrophils) and from bronchoalveolar fluid (BAL) (active neutrophils) in horses suffering from COPD.

Neutrophils were isolated from blood and from bronchoalveolar fluid and spin labelled with MeFASL(10,3) according to standard procedure (Šentjurc *et al.,* 1998). Their EPR spectra were measured at room temperature on Bruker ESP(300) X-band EPR spectrometer. The EPR spectra together with the best fits of computer simulated spectra to the experimental ones are presented in Fig. 3A, whereas Fig. 3B shows the corresponding components of the spectra by which the best fits are obtained. From the experimental spectra it would be difficult to get any quantitative data about the ordering and dynamics in the membrane without computer simulation. We could say only that the membrane of dormant neutrophils is slightly more rigid that the membrane of active neutrophils. However, by computer simulation comparing the spectral components (Fig. 3B) and the parameters by which the best fits are obtained (Tab. 2) we can see that the differences are quite large. In blood there are two domains with order parameter above 0.65 (D1 and D2 in Fig. 3, left side), while in the active neutrophils there is only one such component (D1 in Fig. 3, right side), and the other becomes very disordered. Correlation between order parameter, polarity correction p_A and line-width W is the same as already observed in liposomes, except for the domain D3 in neutrophils from BAL with $S = 0.03$ where spin probe seems to be in very polar environment. One possible explanation is that this component of the spectrum belongs to some micellar aggregates in the BAL samples, what should be examined in further experiments.

Beside a significant decrease of order parameter of all the domains in the membrane, upon activation also the proportion of the most ordered domain (D1) decreases (Tab. 2). The observed fluidisation of the neutrophil membranes upon activation indicates that beside rearrangement of lipids and proteins in the membrane, the composition of lipids in the membrane changes too. This could be connected with extensive deacyilation of arachidoniyl-containing phospholipids, which is observed upon stimulation of neutrophils (Tessner, Greene & Wykle, 1990).

CONCLUSION

In this article we presented the analysis of the EPR spectral line-shapes based on the computer simulation of the EPR spectra with the evolutionary optimization. The superposition of several components was explained in terms of membrane regions with different physical properties. It was shown that the computer-simulation-based EPR spectra characterization reveals the data about the ordering and dynamics in membrane domains as well as the polarity in the environment of spin probe and the distribution of spin probes in the domains. According to these results some information about the rearrangement of molecules in the membrane can be obtain as a result of different factors, which influence the membrane properties. The demonstrated examples try to show the advantages of this method in comparison to conventional procedure, which only gives information about an average fluidity characteristics of the membrane.

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