CHOLESTEROL RICH DOMAINS ARE ESSENTIAL FOR INTERACTION OF LIPOSOMES WITH CELLS

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Cholesterol is a molecule, which plays essential structural role in eucaryotic cells; which includes 30 – 50 mol % of cholesterol. It increases the orientational order of membrane lipid chains and decreases membrane permeability while maintaining membrane fluidity. When added to the phospholipids in model membranes at concentrations higher than 30 mol % it maintains the membrane in liquid ordered state through the large temperature range and in this way protects the cell membrane from drastic permeability changes, which could occur at phase transitions from solid to liquid phases [Bloom, M. Physics in Canada 48 (1992) 7]. As the biological membranes are heterogeneous with the regions with different modes of molecular motions (domains) cholesterol distributes within these domain according to their dynamic characteristics. It prefers to distribute in the regions with saturated hydrocarbon chains and forms separate liquid ordered phases, which coexist with liquid-disordered phases. Cholesterol is essential for the maintenance of these domain types, which determine the boundaries for lateral diffusion of proteins in each phase. By modulating lipid concentrations and external conditions the domains could merge together, what may play the role in regulating membrane properties. In combination with sphingolipids it forms special types of liquid ordered domains – rafts. The role of lipid rafts is to segregate and concentrate membrane proteins and are involved in sorting and distributing lipids and proteins to the cell surface [Simons, K. and Ikonen, E. Science 290 (2000) 1721]. With respect to the mentioned role of cholesterol in biological membranes it is important to study how cholesterol is distributed within the membrane and how this distribution between membrane domains influences the interaction of membranes with cells and proteins.

In this work the changes in the domain structure of liposomes, models of cell membranes, with cholesterol concentration will be measured. The electron paramagnetic resonance (EPR) with the spin probe methylester of palmitic acid (MeFASL(10,3)) will be used. The information about the membrane domain structure will be obtained by computer simulation of the EPR spectra line-shape, taking into account that the membrane is heterogeneous, composed of several membrane domains with different motional characteristics. To obtain best fit of calculated to the experimental spectra the evolutionary optimization method (HEO) was used. It combines stochastic and population-based genetic algorithm, which is good at finding promising regions in complex search space, with Simplex Downhill for fine-tuning. In order to get a reasonable characterization...

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of membrane domain structure multi-run HEO optimization was used together with a newly developed GHOST condensation procedure. According to this method 200 independent HEO simulation runs for each EPR spectrum were applied, taking into account 4 different motional modes of spin probe. The parameters of the best fits were presented by two-dimensional cross-section plot, with order parameter $S$ on abscissa and polarity correction factor $p_A$ on ordinate (GHOST diagrams) [Strancar, J.K. et al. J. Chem. Inf. Model. 45 (2005) 394]. From these plots information about the membrane domain types, dynamic of motion and ordering within the domain types as well as about the polarity of spin probe surrounding can be obtained. Besides, the relative proportion of a particular spectral component is determined. It describes the relative amount of the spin probes with particular motional mode and depends on the distribution of the spin probe between the domain types as well as on distribution and position of the spin probe within the domain. It should be stressed that the lateral motion of the spin probe is slow on the time scale of EPR; therefore an EPR spectrum describes only the properties of a spin label's nearest surrounding. (The computer simulation procedure is implemented in the software package EPRSIM (http://www.ijs.si/ijs/dept/epr/)).

Two examples will be presented: role of cholesterol-rich liposome membrane domain structure in the interaction of liposomes with cells and for the activity of ostreolysin, the cytotoxic protein from oyster mushroom.

**Role of cholesterol-rich liposome membrane domain structure in the interaction of liposomes with cells**

Interaction of alkylphospholipid liposomes with breast cancer cells was measured using electron paramagnetic resonance method (EPR) with advanced characterization of EPR spectra. In our experiments alkylphospholipid octadecyl-(1,1-dimethyl-4-piperidino-4-yl)-phosphate (OPP) which is physiologically active lipid derivative that is very effective in the therapy of experimental human breast cancer, was used. The liposomes were composed of OPP, negatively charged dicetylphosphate (DCP), and cholesterol ranging from 29.4 mol % (N5) to 55.6 mol % (N15) of total lipids. The domain structure of liposomes was measured by EPR at different concentration of cholesterol and compared to the results of liposome-cell interaction measurements. We have found that fusion of alkylphospholipid liposomes with tumor cells depends substantially on the amount of cholesterol in liposome membrane. Liposomes with low amount of cholesterol (below 50 mol %) fuse readily, whereas liposomes with higher cholesterol amount do not fuse. This coincides with the presence of disordered mode of alkyl chain motion in liposomes with low cholesterol amount, which disappears at higher amount of cholesterol.
Role of cholesterol-rich liposome membrane domain structure for the activity of ostreolysin, the cytotoxic protein from oyster mushroom

Ostreolysin (Oly) is a cytolytic 15 kDa protein from edible oyster mushroom (Pleurotus ostreatus). It was found that cytotoxicity of Oly correlates with CHO cell cholesterol contents and Oly's partition in the detergent resistant membranes -rafts (DRMs). Moreover, it binds to supported monolayers and permeabilizes sonicated lipid vesicles (SUV) only if cholesterol (less efficient is ergosterol) is combined with either sphingomyelin or di-palmitoylphosphatidylcholine (DPPC). Addition of mono- or di-unsaturated phosphatidylcholine dramatically reduces Oly binding and consequently pore-formation. This indicates that its activity is strongly dependent of membrane domain structure and is connected with the formation of liquid ordered domains [Sepcic, K. et al. FEBS Lett. 575 (2004) 81]. In this work the membrane domain structure of sonicated vesicles composed of varying proportions of cholesterol and sphingomyelin was investigated and correlated with Oly activity.

It was found that at 25°C the major change in lateral domain structure was observed between 30 and 40 mol % of cholesterol vs. sphingomyelin, which is reflected in significant increase of the most ordered domain and appearance of a new liquid ordered domain. The results coincide with ostreolysin binding and permeabilization of the same vesicles at the same amount of cholesterol and coincide also with phase diagram, which was obtained by fluorescence method.