A Comparative Study of Microscopic and Macroscopic Parameters of Lipid Bilayers

T. Hianik[1], A. N. Tichonov[2] and V. A. Tverdislov[2]

1 Department of Biophysics, Faculty of Mathematics an Physics, Comenius University, Mlynská dolina F1, 842 15 Bratislava, Czechoslovakia
2 Department of Biophysics, Faculty of Physics, Moscow State University, 119 899 Moscow, USSR

A wide scale of various methods is being employed in modern studies of physical properties of biological and model bilayer lipid membranes. Two different groups of methods can be distinguished: those approaching macroscopic membrane parameters, such as viscoelastic properties and surface tension (Passechnik and Hianik 1977; Wobschall 1971), and those disclosing microscopic parameters, such as spin or fluorescent labels (Schreirer et al. 1978; Vladimirov and Dobretsov 1980). A knowledge on the exactness of correlations between various parameters and other properties is required to get an overall idea on the membrane structure and molecular movements within it.

This has stimulated our experiments and a comparative analysis of results obtained in a study of viscoelastic properties of bilayer lipid membranes (BLM) (the macroscopic parameter) with those obtained in a study of lipid vesicles using the method of ESR and a spin label (the microscopic parameter). The viscoelastic properties of BLMs were measured in direction perpendicular to the membrane surface, as characterized by both the Young modulus $E_\perp$ and the dynamic viscosity coefficient η. Essentially, the above parameters reflect the compressibility of the membrane hydrophobic region. BLMs were prepared according to the method of Mueller et al. (1962) on a circular hole ($d \sim 0.5$ mm) in the hydrophobic wall of a teflon cup. The electrolyte used was 0.1 mol/l NaCl. Alternating electrical voltage $U = U_0$ in $2\pi ft$, with an amplitude $U_0 = 140$ mV and a frequency $f = 1000$ Hz, was applied to the membrane. The parameters $E_\perp$ and η respectively, were estimated according to the methods of Passechnik and Hianik (1977) and Hianik et al. (1984). Spin probe ESR spectra were recorded with a Varian E-4 spectrometer (USA) with following settings: microwave power 10 mW, modulation amplitude 0.1 mT, scan rate 2.5 mT/min. A hydrophobic spin label I (12,3) (Syva, USA) was used (for the structural formula, see Fig. 2a). The label (2×10^{-4} mol/l) was added to egg lecithin vesicles (Kharkov Plant of Chemical Preparations, USSR) containing various concentrations of cholesterol. Vesicles were prepared according to the method of Vladimirov and Dobretsov (1980) in a concentration of 3 mg/ml in 0.1 mol/l NaCl + 10^{-3} mol/l EDTA. All experiments were performed at 20 °C.
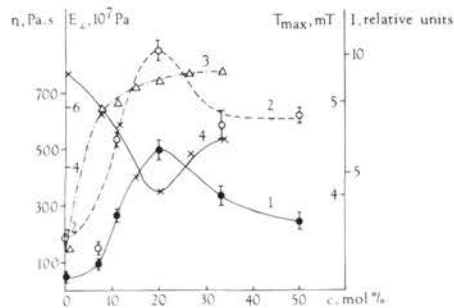
Fig. 1. Relationship between cholesterol concentration and $E_\perp$ (1), η (2), for BLM, and T_{max} (3) and I (4) for egg lecithin vesicles, respectively (for explanation, see the text and Fig. 2).

Fig. 1 shows the relationship between cholesterol content in egg lecithin BLM in n-heptane (20 mg/ml) and $E_\perp$ (curve 1) and η (curve 2). Both curves show a maximum in the cholesterol concentration range of 20 mol %. In a previous work (Hianik et al. 1984) this result was explained as due to clustering in lecithin + cholesterol bilayers. In the concentration range of cholesterol up to the maximum of curve 1 we have two types of clusters: lecithin alone (L) with elasticity modulus E_L, and lecithin + cholesterol (LC) with elasticity modulus E_{LC}. The concentration of LC clusters gradually increases and reaches saturation at the maximum of curve 1. The increasing of the resulting elastic modulus of the whole membrane $E_\perp$ is connected with different value of E_L and E_{LC} ($E_{LC} > E_L$), because as was shown by Passechnik and Hianik (1979) the elastic modulus $E_\perp$ can be defined as follows:

$$1/E_\perp = s_L/E_L + s_{LC}/E_{LC},$$

when s_L and s_{LC} are relative areas of the clusters of pure lecithin and lecihin + cholesterol mixture, respectively. Analogously we can define viscosity coefficient η which is connected with $E_\perp$ as follows:

$$\eta = \frac{E_\perp \cdot \sin \varphi}{2\pi f},$$

where φ is the phase shift (see Hianik et al. 1984). At further increasing of cholesterol concentration the membrane is composed of LC clusters and pure cholesterol (C) clusters with elasticity modulus E_C. In this case, $E_C < E_{LC}$ and analogously as in the above mentioned expression the elasticity modulus of the whole membrane decreased when cholesterol concentration increased ($c > 20$ mol %). The maximum values of $E_\perp$ and η depending on the membrane cholesterol content are another proof of the fact that the interaction forces between molecules in an egg lecithin + cholesterol mixture are greater than in the phases considered independently (see Ivkov and Berestovsky 1981). The results obtained are in accordance with the paper by Pink et al. (1981), in which the phase
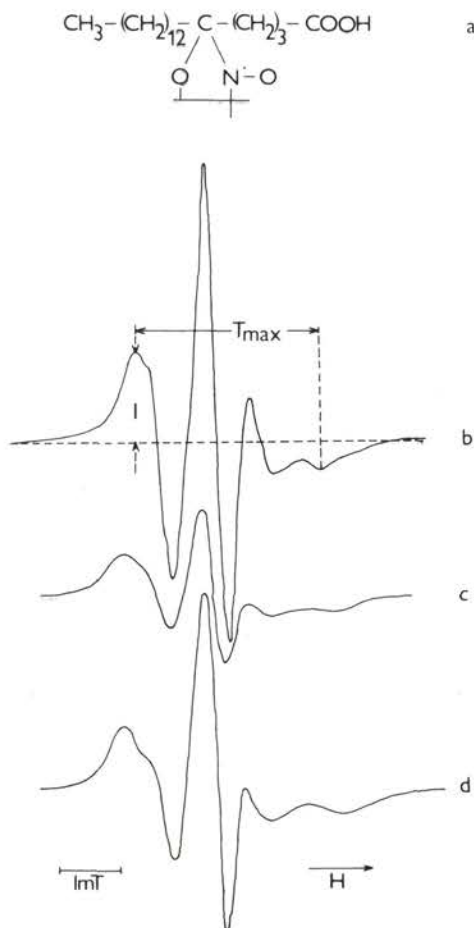
Fig. 2. *a*) Structural formula of the spin label I (12,3); ESR spectra of spin labeled egg lecithin vesicles with various cholesterol contents; *b*) pure lecithin; *c*) cholesterol 20 mol %; *d*) cholesterol 30 mol %.

distribution with maximal values at 20 mol % cholesterol in DPPC (L — β, γ — dipalmitoyl — α phosphatidylcholine) vesicles was determined theoretically on the basis of Raman spectra measurements.

Identical experiments were run using spin labeled egg lecithin vesicles with various cholesterol contents. Fig. 2 shows spectra of I (12,3) for cholesterol-free vesicles (*b*), vesicles containing 20 mol % (*c*) or 33 mol % (*d*) cholesterol. Both the spectrum shape and parameters became changed with the changing cholesterol concentration. Fig. 1 shows the relationship between both T_{max} (curve 3) and intensity I (curve 4) of one of the spectrum components (see Fig. 2*b*), and the cholesterol concentration. Obviously, T_{max} slowly increases as the cholesterol

concentration increases, and gradually reaches saturation. Increasing of T_{max} with cholesterol concentration is due to increased average ordering of the whole membrane, predominantly in the region of the polar head groups of lipids (Schreirer et al. 1973; Smith and Butler 1976; Chapman 1983). This is just the region of the localization of N-oxyl group of I (12,3) spin label (see Fig. 2a). On the other hand, the parameter I does not change monotonously. A minimum is observed in the cholesterol concentration range of 20 mol % (curve 4). This change in the spectrum intensity is probably associated with a distribution of the spin label between L and LC, or LC and C type clusters, which are characterized by different homogeneity of the structure. The region of 20 mol % cholesterol content corresponds to the maximal inhomogeneity of the membrane. The increase of the parameter I for $c > 20$ mol % cholesterol probably shows that the spin label is localized predominantly in cholesterol clusters, in the region of a higher homogeneity as compared to the situation at $c = 20$ mol %. Thus, the parameter I can be compared with $E_\perp$ and η, which characterize the inner region of the lipid bilayer. The latter region is less ordered than that of the polar head groups (Chapman 1983). Due to different size of the hydrocarbon chains of lecithin and cholesterol molecules this region more expressively characterizes the inhomogeneity of the membrane.

In addition, the distribution mentioned could due to different phase states of the system which is characterized by different temperatures of the phase transition (see Lee 1983).

A different distribution of the spin label in a cholesterol containing bilayer was observed earlier (see Smith and Butler 1976). With increasing cholesterol concentration a decreased spin label distribution was observed in bilayers from egg lecithin and dioleoyl lecithin, and an increased distribution in dipalmitoyl lecithin bilayers. Our results have shown that different distributions of spin label are possible in one standard egg lecithin bilayer system at different cholesterol contents.

The present results can thus be summarized as suggesting the existence of a correlation between viscoelastic and ESR parameters of the BLM. This has been suggested by the observation of clusterization occurring in the system of egg lecithin and cholesterol and by the fact that the distribution of the spin label in the membrane depends on the physical state of the membrane which can be characterized by elastic modulus and viscosity coefficient.

References

Chapman D. (1983): Biomembrane fluidity: The concept and its development. In: Membrane Fluidity in Biology (Ed. R. C. Aloia), v. 2, pp. 5—42, Acad. Press, New York

Hianik T., Kvasnička P., Štroffeková K. (1984): Mechanical properties of lipid bilayers with varying cholesterol content. Stud. Biophys. **100**, 23—32

Ivkov V. G., Berestovsky G. N. (1981): Dynamic Structure of Lipid Bilayer. Nauka, Moscow (in Russian)

Lee A. G. (1983): Lipid phase transitions and mixtures. In: Membrane Fluidity in Biology (Ed. R. C. Aloia), v. 2, pp. 43—88, Academic Press, New York

Mueller P., Rudin D. O., Tien H. Ti, Wescott W. C. (1962): Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. Nature **194**, 979—980

Passechnik V. I., Hianik T. (1977): Elastic properties of bilayer membranes in the direction perpendicular to the membrane plane. Kolloid. Zh. **39**, 1180—1185 (in Russian)

Passechnik V. I., Hianik T. (1979): Modification of lipid bilayer elastic properties by different action. Biofizika **24**, 460—465 (in Russian)

Pink D. A., Green T. J., Chapman D. (1981): Raman scattering in bilayers of saturated phosphatidyl cholines and cholesterol. Experiment and theory. Biochemistry **20**, 6692—6698

Schreirer S., Marsh D., Dugas H., Schneider H., Smith I. C. P. (1973): A spin probe study of the influence of cholesterol on motion and orientation of phospholipids in oriented multibilayers and vesicles. Chem. Phys. Lipids **10**, 11—27

Schreirer S., Polnaszek C. F., Smith I. C. P. (1978): Spin labels in membranes. Problems in practice. Biochim. Biophys. Acta **515**, 395—436

Smith I. C. P., Butler K. W. (1976): Systems of oriented lipids as model membranes. In: Spin Labeling. Theory and Applications (Ed. L. J. Berliner), pp. 444—488, Acad. Press, New York, San Francisco, London

Vladimirov Yu. A., Dobretsov G. E. (1980): Fluorescent Probes in Investigation of Biological Membranes. Nauka, Moscow (in Russian)

Wobschall D. (1971): Bilayer membrane elasticity and dynamic response. J. Colloid Interface Sci. **36**, 385—396