

## Elasticity, Strength and Stability of Bilayer Lipid Membranes and Their Changes Due to Phospholipid Modification

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**Abstract.** Elasticity measurements of bilayer lipid membranes (BLM) based on registration of the third harmonic of the membrane current during the application of a periodic tension to the membrane was used to study the effects of lipid peroxidation (LPO) and phospholipase A on BLM. LPO resulted in decreased values of the Young modulus for BLM, while some products of LPO and phospholipid hydrolysis (linolenic acid) were able to increase drastically the modulus. The presence of individual products of LPO and phospholipid hydrolysis in BLM produced non-additive effects on the elasticity, strength and stability of BLM. Lysolecithine strongly affected both the strength and stability of BLM, without changing its elasticity modulus. It was found that the lower the rate of structural changes in lecithine BLM, the longer its lifetime. Membranes having a heterogeneous polar composition form more stable BLM as compared to chemically homogeneous membranes.

**Key words:** Elastic properties — Bilayer membranes — Phospholipid modification — Bilayer stability — Lipid peroxidation

### Introduction

Under physiological conditions, lipid peroxidation (LPO) and phospholipase-induced phospholipid hydrolysis occur in strict succession, eventually resulting in degradation of cell membranes (Schewe et al. 1977). Evidence for structural changes underlying membrane degradation may be obtained from the study of elasticity of membranes containing LPO and phospholipase products (Karagodin et al. 1978). We investigated changes in two parameters of membranes, namely (1) the value of the Young modulus, and (2) the rate of its alteration. The aim of the present work was to see whether there was a correlation between these changes and the stability of bilayer lipid membranes (BLM).

## Materials and Methods

The elasticity of BLM in vertical direction to its plane is described in terms of the Young modulus,  $E_1$ , characterizing the bilayer ability to decrease its thickness upon the application of a compressing pressure,  $p$ . Such a pressure may be produced by applying an electric tension,  $U$ , to the membrane. In order to measure  $E_1$  by the method of Passechnik and Hianik (1977) an alternating electric tension,  $U = U_0 \sin 2\pi ft$ , with a frequency,  $f$ , and amplitude,  $U_0$ , was applied to BLM resulting in membrane compression with a pressure

$$p = C_s U^2 / 2h,$$

where  $C_s$  is the specific capacity of the membrane, and  $h$  is the thickness of its hydrophobic part. The membrane capacity depends on the electric tension according to the rate

$$C = C_0(1 + \alpha U^2),$$

where  $C_0$  is the capacity of BLM at  $U=0$  and  $\alpha$  is the non-linearity index. Due to the non-linear dependence of capacity versus tension, the current passing through the membrane,  $i = d(CU)/dt$ , contains a harmonic with a frequency of  $3f$ , the amplitude of which is determined by the value of the Young modulus for BLM:

$$E_1 = \frac{3}{4} \cdot \frac{C_s \cdot U_0^2}{h} \cdot \frac{A_1}{A_3} \quad (1)$$

where  $A_1$  and  $A_3$  are amplitudes of the main first and third harmonics of the current, respectively.

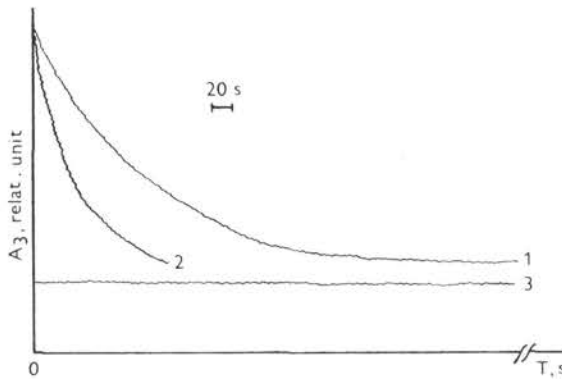
The method of measurement of the mechanical properties of modified BLM, developed in our laboratory, provides a new approach for solving the problems of LPO. In fact except for measurements of the electromechanical stability of BLM there are no other methods to measure mechanical properties of modified membranes and no similar literary data are available.

Measurements were performed at  $U_0 = 140$  mV, the modulus was determined at a frequency of 1262 Hz. BLM were prepared according to Mueller from a lipid solution in *n*-heptane on a round hole 0.5 mm in diameter, bored in the wall of a teflon vessel. The value of  $E_1$  was calculated setting  $C_s = 0.39 \mu\text{F}/\text{cm}^2$  and  $h = 4.9$  nm (Requena et al. 1975). In all experiments, lipids were modified with LPO products in the tested membrane-forming solution prior to BLM formation. Tris-HCL (5 mmol/l) was used as the electrolyte on both sides of BLM (pH 7.4; 20 °C). Egg lecithine (L) was obtained by the standard procedure; membrane fractions from rabbit skeletal muscle sarcoplasmic reticulum (SR) were isolated by the method of Martonosi (1968). LPO in SR membrane suspension was induced by incubation with  $\text{Fe}^{2+}$ /ascorbate system (0.01 and 0.3 mmol/l, respectively) for 60–90 min. Carbonyl derivatives of LPO were determined colorimetrically using thiobarbituric acid. Lipid degradation products were assayed by UV spectroscopy (diene conjugate products) and by thin-layer chromatography in a chloroform-methanol-water system. Lipid hydrolysis was performed by incubating the lipid suspension with phospholipase  $A_2$  (PA) (Calbiochem).

Lysolecithine (LL) was purchased from Serva, linolenic acid (LA) and 2.5 — heptadiene acid (HDA) were of the purest grade available (Reachim, USSR). Other reagents were of the purest or analytical grades. In each series of experiments  $E_1$  was measured in 8–22 membranes, namely: SR — 22 membranes; SR-LPO-18; L-17; L+LA-8; L+LL+LA-8; L+PA-10; L+LL-10; L+HDA-10; L+LPO-9; L+LL+HDA-10; and mean quadratic errors were calculated.

## Results

Alternating current was applied to membranes after about 10 minutes following

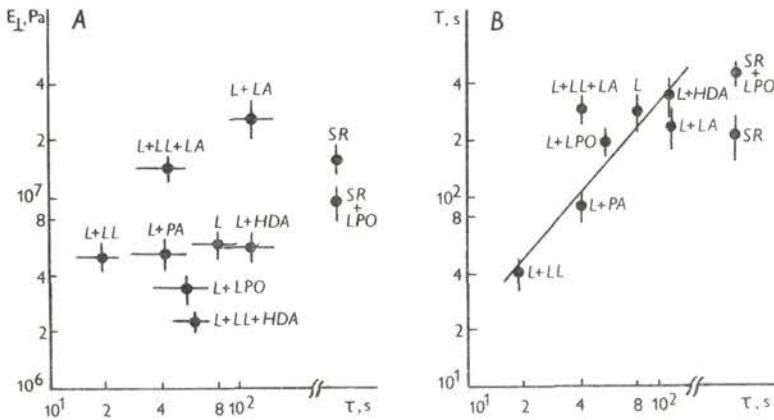


**Fig. 1.** Time dependence of the third harmonic of the membrane current  $A_3$ . BLM composition: 1 — lecithine, 2 — lecithine + lysolecithine, 3 — SR lipids.

blackening of BLM. A gradual decrease of  $A_3$  was observed (Fig. 1); the changes of  $A_1$  were insignificant, the value of the Young modulus,  $E_{\perp}$ , showing a steep rise with time in accordance with the expression(1). The kinetics was characterized in terms of time,  $\tau$ , during which the value of  $E_{\perp}$  increased about  $\sqrt{2}$ -fold. This value represents the time necessary for structural transformations of the membrane to occur after the application of a compressing pressure, and it is a function of the BLM strength (see below). The life-time,  $T$ , measured from the onset of the tension till BLM disruption was taken as the membrane stability index.

Our measurements of the electric membrane capacity using first current harmonic:  $A_1 = U_0 C \omega$  ( $\omega = 2\pi f$ ), as well as results of thickness measurements of bilayers composed of egg lecithin-lysolecithin mixtures by Ivkov and Berestovsky (1981) have indicated small changes of  $C_s$  for different lipid compositions. Thus the value of  $E_{\perp}$  provides a better information than does  $C_s$ .

Fig. 2 A shows the relationship between  $E_{\perp}$  and  $\tau$ ; the value of  $E_{\perp}$  was calculated from the steady-state value of  $A_3$ ; the correlation between  $T$  and  $\tau$  is shown in Fig. 2B, the correlation coefficient being about 0.74. The membranes formed from PA — treated lecithine had values of  $E_{\perp}$ , which differed insignificantly from those for BLM of non-treated lecithine  $E_{\perp L}$ , while the values of  $\tau$  and  $T$  were decreased by a factor greater than 3. In these experiments, hydrolysis resulted in a conversion of 50% of lecithine to lysolecithine. The presence of a maximum possible amount of LL in BLM still causing no appreciable damage to artificial membrane permeability (Kitagawa et al. 1976), i. e. L:LL = 5:1 (here and further molar ratios are given), did not practically change  $E_{\perp}$ , but caused a sharp drop of both  $\tau$  and  $T$ . By contrast, linolenic acid in equimolar amounts resulted in drastic



**Fig. 2.** Correlation of  $E_{\perp}$  (A), and  $T$  (B) to  $\tau$  of bilayer membranes up on lipid modification. BLM composition: L — lecithine; L+PA — phospholipase A-treated lecithine; L+LL — lecithine + lysolecithine; L+LL+LA — lecithine + lysolecithine + linoleic acid; L+LA — lecithine + linolenic acid; L+LPO — lecithine subjected to LPO; L+HDA — lecithine + heptadiene acid; L+LL+HDA — cithine + lysolecithine + heptadiene acid; SR — sarcoplasmic reticulum lipids; SR+LPO — sarcoplasmic reticulum lipids subjected to LPO.

rise of  $E_{\perp}$  (4.77 — times that of  $E_{LL}$ ). The simultaneous presence of both lecithine hydrolysis products in BLM in concentrations as in previous experiments gave rise to the formation of membranes having intermediate values of  $E_{\perp}$  and  $\tau$  as well as of  $T$  of the same order of magnitude as in the case of lecithine BLM. It should be noted that, for the former BLM, the value of  $\tau$  practically coincided with that for PA-treated lecithine membranes. LPO products (100 nmoles of hydroperoxides per mg of L and 21 nmoles of malonic dialdehyde per mg of L) „softened“ the membrane and decreased the  $T$  and  $\tau$  values. BLM the composition of which may be considered as a model of LPO-treated membranes (L : LL : HDA = 5 : 1 : 1) had a several times lower value of  $E_{\perp}$  than that of  $E_{LL}$ .

The study of BLM prepared from SR lipids has shown that the value of  $E_{\perp}$  was 3 times as high as that of  $E_{LL}$ , the value of the Young modulus being practically time-independent. The life-time of such membranes was increased under the action of LPO, while the value of  $E_{\perp}$  showed a slight decrease.

Our measurements were performed at  $U_0 = 140$  mV. It has been shown earlier that variation of  $U_0$  had a very small influence on the value of  $E_{\perp}$  (Passechnik and Hianik 1979).

Thus, for lecithine BLM a correlation between  $T$  and  $\tau$  may be observed; i. e.  $T$  increases with  $\tau$ . For SR lipid membranes having a very high value of  $\tau$ , no such correlation is found. A comparison of the data presented in Figs. 2A and 2B suggests that there probably is no direct correlation between the membrane life-time and Young modulus values.

## Discussion

Prior to come to considerations of concerning possible mechanism of action of LPO products and phospholipase A, it seems necessary to understand the physical sense of  $E_{\perp}$ ,  $\tau$  and  $T$ . BLM prepared from various lipids with n-heptane contain approximately 60% of the solvent (Fettiplace et al. 1971) and possess a complex structure (Passechnik 1980). The membrane plane contains flat areas and microheterogeneities, the latter being proposed to be metastable structures. They are generated during the bilayer formation because the solvent has no possibility to leave the membrane and it should be packed somewhere in it. The diameter of microheterogeneities approximately equals the bilayer thickness  $h$  and their height is several times as large as  $h$ ; in this case, these structures do almost not affect the membrane capacity. The flat areas also are of heterogenous structure and their sections appear as a „sandwich“ consisting of two rigid layers with an intermediate layer with a low value of the Young modulus and a high content of the solvent, the latter determining the value of  $E_{\perp}$ ; the value of  $E_{\perp}$  is independent of the microheterogeneities, but it linearly depends on the solvent concentration in the bilayer: the higher the concentration, the smaller the value of  $E_{\perp}$  (Hianik 1979). Thus, the value of  $E_{\perp}$  is an index of elasticity of the inner BLM layer.

The time  $\tau$  reflects changes in the equilibrium between the lipids and the solvent within the flat areas of BLM. The application of a pressure results in a shift in the equilibrium towards the release of the free solvent which migrates to the microheterogeneities from the flat areas, thus increasing the elasticity modulus of the latter with time (or decreasing  $A_3$ ) as observed experimentally (Fig. 1). The time of this process,  $\tau$ , may depend on two factors; on the rate of the bilayer structural changes and on the time of diffusion of the released solvent into the microheterogeneities. The diffusion time is believed to be very short, since the solvent molecules have to cover the distance of a nm order; therefore, in this case the limiting factors is the time of structural transitions. This value is an indirect index of the bilayer strength, because, at a great strength of BLM, their elasticity does not change under the given values of the tension applied and the value of  $\tau$  can thus be regarded as a strength index of the BLM flat areas. The correlation between  $\tau$  and the membrane life-time,  $T$ , for egg lecithine shown in Fig. 2B, confirms the assumption that  $T$  is an index of strength of the bilayer flat areas.

We failed to measure the value of  $\tau$  in SR lipid membranes (Fig. 1); the lack of kinetics is also typical of membranes with a low solvent content, e. g. egg lecithine BLM in n-hexadecane (Passechnik and Hianik 1979). Such membranes are apparently different from lecithine membranes in n-heptane in their ability to withstand deforming forces.

In order to analyze the results of simultaneous action of several factors on BLM, let us consider the hypothetical values of its parameters for a situation when

the entire membrane consists of non-interacting areas having different compositions. If there are two types of such areas with parameters  $E_1$ ,  $T_1$ ,  $\tau_1$  and  $E_2$ ,  $T_2$  and  $\tau_2$ , the corresponding parameters for the membrane as a whole are:

$$1/E_{\perp} = (1 - s)/E_1 + s/E_2 \quad (2)$$

$$1/T = (1 - s)/T_1 + s/T_2 \quad (3)$$

$$1/\tau = (1 - s)/\tau_1 + s/\tau_2, \quad (4)$$

where  $s$  is the portion of the second type areas. Equation (2) was introduced by Passechnik and Hianik (1979), equation (3) follows from the law of summing the probabilities of stochastic events, i. e. ruptures of individual sites of BLM, while expression (4) is an approximating equation following from the correlation between the  $\tau$  and  $T$  values (Fig. 2B). It follows from the above expressions that, under the given experimental conditions, the parameters of the whole membrane are characterized by intermediate values, between the parameters of its individual sites.

Let us now consider the effects of various LPO products and phospholipase A. Of the two fatty acids linoleic and heptadiene investigated, only the former increased  $E_{\perp}$  significantly. Presumably the long rigid acyl chain of LA (18 carbon atoms) considerably stabilized the inner layer, while the short chain of HDA cannot simply „reach“ it. It should be noted that the detergent, acetyltrimethylammonium, the chain of which contains 12 carbon atoms, retains its ability to change the properties of the inner layer by increasing its  $E_{\perp}$  value (Passechnik and Hianik 1979).

When the membrane contains both one of these fatty acids and lysolecithine, the latter does not form separate areas. Indeed, it follows from equations (2) — (4) that the membrane parameters should be expected to have intermediate values as compared to the membranes prepared of L + LL or L + LA (for linolenic acid). However, this could not be observed experimentally (Fig. 2), e. g. the value of the Young modulus was lower for BLM prepared of an L + LL + HDA mixture than that for L + LL or L + HDA mixtures. The mechanism underlying decrease of the  $E_{\perp}$  value in L + LL + HDA membranes as compared to the value of  $E_{LL}$ , and a simultaneous slight change of the  $\tau$  values are not clear: LL destroys the structure of the inner part of the bilayer, and the short-chain HDA cannot make it firmer; it might thus be expected that a combined action of these two agents results in the formation of a more „loose“ structure with lower values of  $E_{\perp}$  and  $\tau$ . This divergence with experimental data can probably be attributed to the ability of LL and HDA to form clusters with an action on the bilayer than different from that of their individual components.

The two model systems used in our studies, L + LL + LA (PA action model) and L + LL + HDA (LPO action model) show no quantitative coincidence with the parameters of the model BLM. In the first case it may be due to the fact that LA is

rather long and rigid and it thus provides for great rigidity of the bilayer structure; in a true bilayer membrane lecithine become hydrolysed in the presence of fatty acids of a smaller length and with fewer double bonds. Correspondingly, the lower value of  $E_{\perp}$  in L + LL + HDA membranes in comparison with that of LPO-treated membranes is probably due to the absence of long-chain fatty acid residues, which are responsible for increased  $E_{\perp}$  values.

The data obtained throw light on some possible causes of the combined action of various lipid products. E. g., the action of lysolecithine formed under the action of LPO and phospholipases on the membranes is compensated by fatty acids, of the increase of  $\tau$  after the addition of LA and HDA. Also, there is a mutual activation of effects of some product combinations, e. g. a significant decrease of the Young modulus at the simultaneous presence of LL and HDA in the membranes. Presumably, certain fatty acids have a specific action and they drastically change the membrane elasticity, similar to LA. These peculiarities of a combined action of some agents were revealed in experiments with egg lecithine BLM.

In our experiments the elasticity of the SR lipid membranes did not change with time. A typical distinctive feature of SR lipids is a heterogeneity of their polar composition, which may account for the increased strength of the inner layer of BLM. It should be noted that both membranes with altered and unaltered kinetics of  $E_{\perp}$  may undergo disruption, thus indicating the existence of different mechanisms of structural transformation of the membrane after the application of a compressing pressure.

Extrapolation to biological membranes of experimental data obtained for model systems demonstrates that, during the membrane structure disassembly, LPO acts as a mechanism which makes the lipid-protein components accessible to both phospholipase and proteases (Schewe et al. 1977). It therefore seems important that the mutual activation of LPO products gives rise to a considerable „softening“ of the membrane the detergent-like action of lysolecithine and fatty acids being realized via various mechanisms. Judging from the changes in the lecithine membrane elasticity, LPO and phospholipase A presumably produce different effects on membrane with different structure of lipid molecules. Finally, the results obtained are essential for the study of reparation processes, since the mutual compensation of phospholipase A products can play an important role in the reparation occurring after the formation of lysoproducts. This may be of importance in the case of severely deforming cells, e. g. erythrocytes.

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