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Short communication

Mechanical Response of Bilayer Lipid Membranes During Bacteriorhodopsin Conformation Changes

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Conformation changes of protein incorporated into a membrane may be accompanied by significant changes in physical properties of their lipid environment. Several questions arise in this respect: 1. Are changes occuring during the functioning of the membrane-incorporated protein limited to the protein itself, or do they concern the whole membrane? 2. Do structurally and mechanically nonequilibrium states of the membrane occur during the process of protein conformation changes? The above questions may be approached using physical properties of the membrane. Of all the membrane physical parameters, the mechanical properties take a special position. They are functionally important and have an integral nature, i. e. they may be employed to study the physical state of the membrane as a whole. Also, a suitable molecular system is crucial to study these problems. Purple membranes of halobacteria, containing an integral protein, bacteriorhodopsin (BR), are one of the systems that may be used for this purpose. It is known that, after lighting purple membranes, conformation changes of bacteriorhodopsin take place (Packer et al. 1977), accompanied by the generation of a membrane potential in case of an asymmetric arrangement of BR in the bilayer lipid membrane (BLM) (Dancsházy and Karvaly 1976). Using the methods of measurement of the Young modulus of elasticity in the direction normal to the membrane surface ($E_{\perp}$), membrane capacitance (C) (Passechnik and Hianik 1977) and dc voltage (U_1) (Carius 1976) we have studied changes of these parameters on BLM in the presence of fragments of purple membranes containing bacteriorhodopsin. The purple membranes were supplied in a lipid solution of asolectin in n-heptane (40 mg/ml w/v) at a concentration of ~75 μg of protein per 1 mg of lipid. BLM were formed of this mixture according to the method of Mueller et al. (1962) on a circular hole ($d \sim 1$ mm) in the wall of a teflon cup filled with a solution of 0.1 mol/l NaCl in monodistilled water. All chemicals used were of chemical purity grade. All experiments were made at the room temperature ($T = 20$ °C).

To measure the values of $E_{\perp}$, C and U_1 according to the above methods, ac voltage with an amplitude of $U_0 = 100$ mV and frequency $f = 1000$ Hz was applied to the membrane. Membranes were prepared from a solution containing BR in

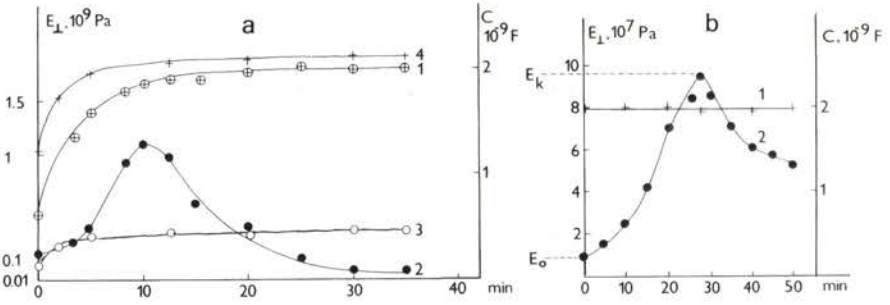

Fig. 1*a*:. Change kinetics of C (curve 1) and $E_{\perp}$ (curve 2) during the formation of bacteriorhodopsin-modified BLM of asolectin in n-heptane (in dark) and change kinetics of $E_{\perp}$ (curve 3) and C (curve 4) during the formation of BLM from asolectin in n-heptane without bacteriorhodopsin. *b*: Change kinetics of C (curve 1) and $E_{\perp}$ (curve 2); membrane prepared of asolectin in n-heptane, modified by bacteriorhodopsin; illumination with white light (10^{-2} W/cm^2). Illumination interval $t = 50$ min.

a light-adapted form. Membrane formation proceeded in dark. Considerable changes in $E_{\perp}$ and C (Fig. 1*a*) could be observed during this process. Changes in the membrane electric capacity (curve 1) showed a usual pattern (cf. Passechnik and Hianik 1979), dependent on the formation of a bilayer membrane, while those in $E_{\perp}$ (curve 2) were biphasic. During the initial ~10 min. $E_{\perp}$ was increasing with a decrease during subsequent ~20 min. gradually reaching a steady state value of ~10^7 Pa. After one hour, when the values of $E_{\perp}$, C and U_1 had been stabilized, the membrane was illuminated with white light (~10^{-2} W/cm^2). During the whole period of illumination, marked changes of $E_{\perp}$ (Fig. 1*b*, curve 2) were observed; these were not accompanied by changes in the electric capacity of the membrane (Fig. 1*b*, curve 1). Due to the symmetric distribution of purple membranes in the BLM, U_1 did not change and remained at a constant level of 4 mV. The kinetics of $E_{\perp}$ changes during the illumination was of irreversible nature and it did not change after switching off the light. Relatively reversible changes of the mechanical properties were observed only during the process of membrane forming, when the membrane with a light of a high intensity was illuminated for very short intervals (~10 s). Simultaneously, the Young modulus was also changing (Fig. 2). All experiments (on 15 membranes) were well reproducible. The mean quadratic errors of measurements of $E_{\perp}$, C and U_1, respectively, did not exceed 20 %.

These results have shown that changes in the mechanical properties of BLM containing purple membranes, are variable, depending on the state of BR. During the membrane formation in absence of light, the marked changes observed in $E_{\perp}$

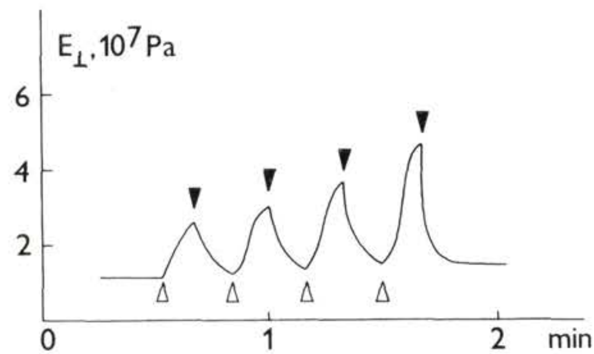

Fig. 2. Change kinetics in $E_{\perp}$; membrane prepared from asolectine in n-heptane, modified by bacteriorhodopsin; repeated by illuminated with 0.1 W/cm^2. △: light switched on; ▼: light switched off.

may have been due to several factors, such as: a) gradual formation of the membrane with the incorporation of purple membranes into the bilayer structure of BLM; b) conformation changes of BR due to alternating electric voltage (electrochromism); c) conformation changes of BR secondary to its transition from the light-adapted to dark-adapted state. Let us analyze the above variants.

a) As shown earlier (Passechnik and Hianik 1979), a nearly threefold increase in $E_{\perp}$ is observed during BLM formation under alternating voltage, with the variable reaching an essentially constant value (Fig. 1*a*, curve 3). Also, the electrical capacity rises to reach a constant value (Fig. 1*a*, curve 4). A comparison of kinetic curves between BR-containing and BR-free BLMs shows an almost identical kinetics of electric capacity changes with markedly differing changes in $E_{\perp}$. Thus, changes associated with the membrane transition to another structural state are predominant.

b) The effect of voltage on BR conformation changes in the membrane is not negligible. E.g., Borisevich et al. (1978) have shown that considerable changes in BR absorption spectra occur under direct voltage. These changes had a time course of ~1 s and were reversible. In our experiments, $E_{\perp}$ and C were recorded under alternating voltage with a frequency of 1000 Hz, i.e. with a characteristic change of 1 ms. The effect of electrochromism may thus not be expected to play a dominant role in the marked changes of BLM $E_{\perp}$ observed. However, this suggestion would require further experimental testing and analysis.

c) Obviously, conformation changes of BR molecules during the transition from the light-adapted to the dark-adapted state is the most likely underlying cause of the marked changes in $E_{\perp}$ during the formation of BR-containing membranes in dark. This suggestion is also supported by typical $E_{\perp}$ change kinetics of the given process ($30 \div 60$ min.), which is identical with time course of the above transition process as recorded using optical methods (Stockenius et al. 1979).

The mechanical response of the membrane during the activity of bacteriorhodopsin have suggested significant structural changes in BLM. Contrary to voltage and current relaxation under approximately similar conditions (Dancsházy and Karvaly 1976) with characteristic times of 1—2 seconds, the time intervals of the mechanical relaxation were as many as 10 minutes or more. Thus, the processes under study are rather slow and they are probably due to extension of BR conformation changes into large regions of the membrane. The membrane gets into a new state characterized by a different value of the elasticity modulus E_k, and $E_k > E_0$, when E_0 is the initial modulus of elasticity. Let us assess the minimum dimensions of the regions changed in the vicinity of a BR molecule in the membrane. As already shown by Passechnik and Hianik (1979), the value of $E_{\perp}$ is an integral characteristic of the membrane. In case of a non-homogeneous membrane, consisting e.g. of two regions with different values of the moduli of elasticity, E_m and E_0, and different relative surfaces, s and $(1-s)$, we can write:

$$\frac{1}{E_k} = \frac{1-s}{E_0} + \frac{s}{E_m}, \tag{1}$$

where E_k is the value of the elasticity modulus of the whole membrane, E_m is the value of the elasticity modulus of areas changed due to conformation changes of BR, and E_0 is the value of the elasticity modulus of unchanged regions (see Fig. 1*b*). In agreement whith this notion, the rise of the modulus of elasticity (Fig. 1*b*) may be interpreted as a gradual increase in the diameter of regions with a changed structure, and by the high value of the modulus of elasticity E_m. The region of the maximum (Fig. 1*b*, curve 2) of $E_{\perp}$ probably reflects mutual overlapping of simultaneously changing regions.

We plotted the dependence of $1/E_k$ on $1/E_0$ in accordance with eq. (1). Parameters E_0 of identically composed membranes may differ from each other and may vary within an interval of one order of magnitude, due to physico-chemical properties of lipid/solvent distribution (Passechnik et al. 1981). Fig. 3 shows linear regression of the dependence of $1/E_k$ on $1/E_0$: $1/E_k = (5.3 \pm 1.1) \times 10^{-2}/E_0 + 1.5 \times 10^{-9}$. The correlation coefficient of 0.85 suggests this dependence to be close to linear and s may be considered constant. However, $s = \text{const.}$ is only relative and it means that the area of the changed membrane structure per one BR molecule is approximately identical as long as the changed regions begin overlapping (the region to the left from the kinetic curve maximum, Fig. 1*b*). However, the areas of the changed structure regions obviously get further changed and they become overlapped (the region to the right from the kinetic curve maximum, Fig. 1*b*).

The plot of the above dependence is a straight line (Fig. 3). From its slope we

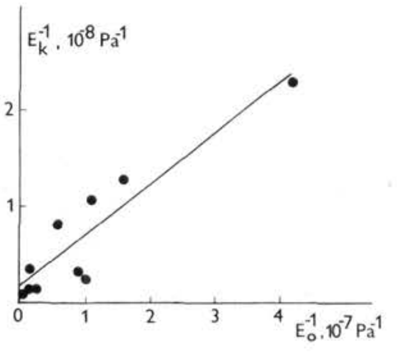

Fig. 3. Dependence of $1/E_k$ on $1/E_0$; membrane prepared from asolectin in n-heptane, modified by bacteriorhodopsin (for explanation, see the text).

can estimate the total relative area of the changed region, $s = 94.7$ % and its elasticity modulus, $E_m = 6.3 \times 10^8$ Pa. The exact determination of the dimension of the changed areas of the membrane would require the knowledge of the number of BR molecules in the membrane. Because of the symmetric distribution of purple membranes in BLM with compensation of H^+ currents, the solution of this question is rather difficult. The known BR concentration in the membrane-forming solution, BLM dimensions and the knowledge that one purple membrane fragment (with a diameter of ~0.5 μm) contains approximately 500 BR molecules with a molecular weight of 26,000 (Stockenius et al. 1979) may be used to estimate the average number of BR molecules in BLM. In our case, there were in average 2.85×10^8 BR molecules in BLM. This means that as soon as regions of changed membrane structure begin overlapping, the diameter of this region will reach ~60 nm. Taking distance between BR molecules in the purple membrane fragment as the lower limit of the region dimensions, we get a value of ~20 nm. Thus the diameters of the changed areas may be supposed to be equal to or more than the distance between individual molecules of bacteriorhodopsin in the purple membrane, i.e. 20 nm or more.

Our results suggest that conformation changes of bacteriorhodopsin in purple membranes result in significant structural changes of BLM. Slow relaxation times of these processes likely indicate that mechanical relaxation of the membrane is one of the mechanisms that can ensure co-operation of the membrane processes as well as utilization of the energy liberated during the exergonic processes (see Blumenfeld 1981, 1983).

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