ELECTRIC BREAKDOWN OF BILAYER PHOSPHOLIPID MEMBRANES UNDER ULTRAVIOLET IRRADIATION-INDUCED LIPID PEROXIDATION

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1. Introduction

Lipid peroxidation (LPO) of unsaturated fatty acids in bimolecular lipid membranes (BLM), induced by ultraviolet irradiation [1–3] or by chemical agents [4], brings about an increase in conductivity of these membranes. For BLM prepared from egg phosphatidylcholine/cholesterol mixture this effect is accounted for by the rise of proton permeability [3,5]; the maximal conductivity enhancement being $\leqslant$2–3-times higher as compared to the initial level [3]. In contrast with this case, BLM prepared from brain phospholipids [2] exhibited a much more pronounced effect: the conductivity rise was sharp and reached several orders of magnitude. In the light of the observation that ultraviolet-induced LPO reduces BLM electric stability (the measure of which can be the 'breakdown' potential) [6], one may assume that the electric instability contributes significantly to the observed 'high amplitude' increase in BLM conductivity caused by LPO. In the LPO reaction a great variety of products is produced [7] and the secondary products of LPO are found to be the most effective in the activation of BLM ionic conductivity [1]. But the precise mechanism by which these substances influence the electric properties of lipid membranes is still to be unravelled. It is the purpose of this paper to estimate the contribution of electric breakdown to the LPO-induced increase in the ionic permeability of BLM and the involvement of charged LPO products into this process.

2. Materials and methods

Lipids were extracted from rat liver mitochondria by the method in [8]. Cholesterol was recrystallized twice from ethanol. BLM were formed from solution of lipids in *n*-heptane (20 mg/ml) in a 1 mm i.d. teflon vessel placed in the thermostated (37–39°C) quartz cuvette. The bathing solutions contained 50 mM KCl and 5 mM Tris–HCl (pH 7.4). The electric circuit for the measurements of BLM resistance and breakdown potential included a standard resistor (10^{10} Ω) and voltage source in series connection. Voltage drop across BLM was monitored with an ED-05M (Soviet) electrometer and recording potentiometer; membrane resistance was calculated for each point of the kinetic curves. LPO was induced by ultraviolet irradiation of BLM through the flat wall of the quartz cuvette. In order to isolate the effective spectral region (250–350 nm) the aqueous solution of $CoSO_4$ and $NiSO_4$ was used as a filter of the light of high pressure quartz mercury lamp (SVD-120A, Soviet). Spectral distribution of the radiation was demonstrated [2]. The total intensity of the radiation was estimated by the ferrioxalate actinometry method [9], and was found to be 0.05 E $.m^{-2}.min^{-1}$.

3. Results and discussion

Typical curves for the changes in BLM resistance under ultraviolet irradiation are presented in fig.1.

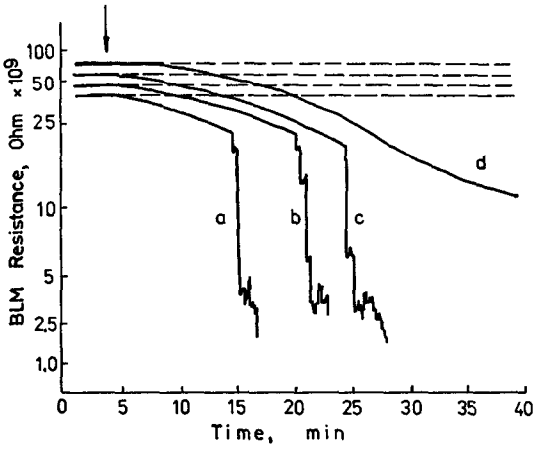

Fig.1. The effect of the potential applied and of cholesterol on ultraviolet-induced decrease in BLM resistance. (a,b,c,d) The values of the potential applied for resistance measurements were 100, 50, 20 and 100 mV, respectively. BLM were formed from mitochondrial lipids (a–c) and from the mixture of these lipids and cholesterol (d) (2:1, w/w). Arrow indicates the onset of irradiation; (- - -) unirradiated BLM.

The values of initial resistance for BLM prepared from various mitochondrial lipid preparations differ by ≤1.5–2-fold. The other factor affecting these values is the voltage applied, in accord with non-linearity of voltage–current curves [6]. One can see that the resistance of BLM prepared from mitochondrial lipids is at first decreasing gradually by a factor of 2 or 3, and then drops suddenly down by several orders of magnitude (fig.1b). Similar curves were obtained with BLM prepared from erythrocyte or rat liver microsome lipids. The slow gradual decrease of BLM resistance under moderate ultraviolet doses might be accounted for by the proton conductivity enhancement [2].

It is very important that the onset of BLM resistance drop depends on the potential applied to the membranes: the higher its value, the smaller the ultraviolet dose at which the drop occurs (fig.1a–c). It seems convincing that the electric breakdown can be the physical base for BLM resistance fall. Apparently, BLM electric breakdown occurs at the point where the decreasing breakdown potential of the membrane goes down to the value of the voltage applied to the membrane. In our experiments the electric breakdown did not immediately cause the rupture of the membranes, since the current was limited by the standard resistor in the circuit. The above conclusion is also consistent with the results of our experiments on the effect of cholesterol which is known to enhance the electric stability of lipid membranes [10,11]. For BLM prepared from mitochondrial lipid/cholesterol mixture the resistance drop was not detected. Small and gradual resistance decrease is typical of such BLM (fig.1d) apparently due to the increase in proton conductivity, as it was observed in BLM prepared from egg lecithin/cholesterol mixture [3].

It was shown [12] that under ultraviolet-induced LPO, negatively charged acidic secondary products were produced (pK ~6.0). On the other hand, it is known that the increase in the net surface charge leads to the reduction of BLM breakdown potential [10,13]. Therefore, the influence of Ca^{2+} and pH on the membrane electric stability was also studied. On the addition of $CaCl_2$ to the medium the decrease in the breakdown potential under ultraviolet irradiation was significantly inhibited (fig.2A) and consequently a sharp decrease in BLM resistance took place under higher irradiation doses (fig.2B). This was most likely the result of the screening of negatively charged LPO products by Ca^{2+}, rather than of other factors like, for instance, the inhibition of LPO process itself. In fact, experiments with liposomes have demonstrated that Ca^{2+} even slightly intensify LPO. The acidification of BLM bathing medium, which resulted in neutralization of charged LPO products on the membrane surface lowered the membrane ultraviolet sensitivity. The ultraviolet irradiation dose producing BLM breakdown at pH 6.0–6.5 was greater than the dose at pH 8.0–8.2 by a factor of 1.5 ± 0.2 (mean ± SD of 8 independent determinations). One may therefore suggest that it is the formation of secondary products which is important for the decrease of BLM electric stability.

Thus, LPO causes the transition of BLM with low content of cholesterol to the state of electric instability as a result of the fall of breakdown potential down to the value of the voltage applied; BLM conductivity being sharply increased. A possible reason of such LPO influence is the formation of secondary LPO products with negatively charged groups. Electric breakdown may contribute to the loss of membrane barrier function not only under ultraviolet irradia-

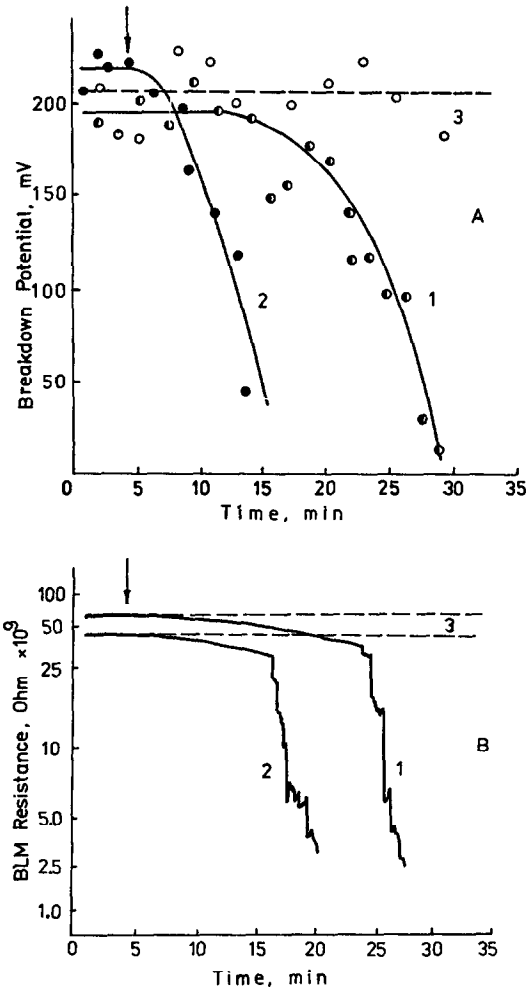

Fig.2. The effect of Ca^{2+} on the ultraviolet-induced decrease in BLM breakdown potential (A), and in BLM resistance (B). (1,2) BLM in media with and without $CaCl_2$ (2 mM), respectively; (3) unirradiated BLM. Arrow indicates the onset of irradiation.

tion, but as well under the action of other factors reducing the electric stability of lipid bilayer. A pronounced increase in BLM conductivity is observed under the action of phospholipase A_2 [14], and on the insertion of lysophosphatidylcholine [15]; the electric breakdown may participate in both cases. The electric breakdown was registered on films of flax-seed oil under γ-irradiation [16].

In the living cell the electric breakdown can be involved in the membrane-destruction processes of different origins, as far as all biological membranes are permanently affected by the membrane potentials which they generate by themselves.

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