Electrical Stability of Artificial Membranes

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Abstract. The electrical breakdown potential of the planar lipid membranes has been shown to decrease following UV-induced lipid peroxidation, action of phospholipase A_2, adsorption of protamine sulphate and expansion of the membrane by hydrostatic pressure. Membrane potential generated upon the addition of potassium acetate (or ammonium sulphate) and protonophore CCCP to liposomes, when large enough, was also able to break membranes; this was suggested by liposome swelling and a rapid decrease in suspension turbidity. UV-irradiation decreased liposomal membrane breakdown potential, while cholesterol increased it. Detergents and water-soluble products of lipid peroxidation decreased the breakdown potential. The possible role of the membrane electrical breakdown phenomenon in cell pathology is discussed.

Key words: Electric breakdown — Barrier function of membranes — Liposomes — BLM — Membrane pathology

Introduction

It is well known that biological membranes lose their barrier functions if the transmembrane potential difference exceeds some critical value. If the potential applied to the membrane is higher than the critical one, membrane permeability (and hence membrane conductance) starts rising dramatically, and increases by several orders of magnitude. This phenomenon has been called membrane electrical breakdown (Zimmermann et al. 1976; Kinosita and Tsong 1977; Abidor et al. 1978). Theories of this process have been developed by several authors (Zimmermann et al. 1976; Kinosita and Tsong 1977 Abidor et al. 1979).

It is obvious that electrical breakdown may occur as a result of both, an increase in the membrane potential and a decrease in the membrane stability. We have recently assumed that the electrical breakdown by the membrane's own potential is a major mechanism of disordered cell function in disease (Putvinsky et al. 1979; Puchkova et al. 1983; Vladimirov et al. 1983).

Still earlier, it was concluded that there should be only four direct causes leading to an increase in the membrane permeability in pathology (Vladimirov

1973): lipid peroxidation, activation of membrane phospholipases, osmotic expansion of cells and cell organelles, and, finally, protein adsorption on the membrane surface (or changes in the conformation of membrane proteins). It was rather surprising that all the four factors led to one final result — a dramatic increase in membrane permeability.

The aim of the present paper was to show that the above four factors can actually decrease the electrical stability of the lipid bilayer. Artificial phospholipid membranes (planar lipid membranes, BLM, and vesicular, liposomes) were used as model membrane systems. Membrane electrical breakdown induced by short pulses of strong electrical field applied to a suspension of membrane particles is now widely used to study membrane electrical stability (Zimmermann et al. 1976; Kinosita and Tsong 1977) and cell inactivation (Hamilton and Sale 1967), membrane fusion and other phenomena induced by electrical breakdown (Scheurich and Zimmermann 1981). However, the pulse technique does not allow the evaluation of the actual role of electrical breakdown in membrane functioning, since potentials in the living cell are of different origin, and have different amplitudes and temporal characteristics.

In the present work it was shown that electrical breakdown can be produced in vesicular membrane by the same potential as in the biological membrane. The measure of membrane electrical stability was the maximal potential which the membrane could bear. It has been established that the critical membrane potential is a definite characteristic of the membrane state and that it changes when the membrane undergoes alterations.

Materials and Methods

Lipids were isolated from egg yolk by the Bligh and Dyer method (Bligh and Dyer 1959) and from liver-mitochondria of albino rats (Hogeboom et al. 1948). Egg lecithin was obtained from the bulk phospholipid fraction by means of thin layer chromatography on silica gel "LS-5/40" (Czechoslovakia).

BLM were formed from a solution of lipids in *n*-heptane (20 mg/ml) on 1 mm (i.d.) hole in the wall of a teflon vessel placed in a thermostated quartz cuvette. The bathing solution contained 50 mmol/l KCl and 5 mmol/l Tris-HCl (pH 7.4). The voltage drop across BLM with a standard resistor (10^{10} Ω) connected in series and a constant voltage source was monitored with an ED-05 M (USSR) electrometer and recording potentiometer.

Liposomes were prepared from egg lecithin by a two-step procedure: first, multilayer lyposomes were prepared by the method of Bangham et al. (1967); the liposomes were then commited by freeze-thawing using nitrogen (Sorokovoy et al. 1974), yielding smaller but still multilayer liposomes.

The permeability of liposomes to ions was assessed from their osmotic behavior. Liposome swelling or shrinkage resulting from the additions of salts were monitored turbidimetrically at 680 nm. Lipid peroxidation in bilayer lipid membranes was induced by UV-irradiation (250—350 nm) (Putvinsky et al. 1977). The samples were exposed to UV light of a SVD 120A quartz mercury lamp of superhigh pressure passed through a water solution of $NiSO_4$ and $CoCO_4$. Suspensions of liposomes were irradiated in quartz cuvettes (1 cm think) under continuous stirring. BLM were irradiated through

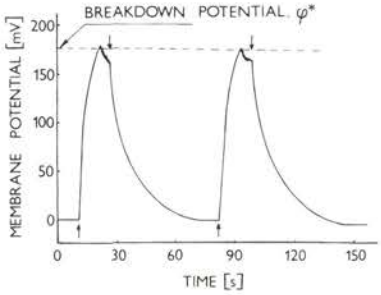

Fig. 1. Time-course of the potential drop on BLM connected in series with an RC element and voltage source, E. After breakdown the circuit is switched off (arrows down) and then switched on after a short internal (arrows up).

a flat front wall of a quartz cuvette. The total intensity of the radiation was estimated by ferrioxalate actinometry method (Parker 1972).

Results

Our experiments with the model lipid membranes was aimed at 1) developing method for estimation of the electrical stability of the lipid bilayer, and 2) detecting changes in electrical stability under the action of various factors. These problems have been solved rather easily in the case of BLM (Smirnov et al. 1981.). The measure of electrical stability of BLM was the maximal potential difference (breakdown potential) which was still tolerated by the membrane (Fig. 1). If a voltage step (of about 1 V) was applied from the source the potential on the BLM was growing rather slowly due to a high RC of the circuit. As soon as the membrane potential has reached a critical value (φ^*) the electrical breakdown started developing and the membrane resistance dropped. Consequently, the potentials in the circuit redistributed so that the membrane potential decreased (Fig. 1). At this moment switching off the circuit prevented mechanical rupture of BLM. The electrical breakdown of BLM was completely reversible and the breakdown potential value could repeatedly be measured, thus enabling to detect changes.

The breakdown potential value φ^* was shown to be a rather labile characteristic. Fig. 2 shows breakdown potential values for BLM following UV-induced lipid peroxidation (LPO) (Fig. 2*a*), the administration of phospholipase A_2 (Fig. 2*b*), following adsorption of the positively charged protein protamine sulfate on the membrane surface (Fig. 2*c*), and following mechanical expansion of the membrane produced by the application of a hydrostatic pressure gradient (Fig. 2*d*). It is obvious that all the four factors studied resulted in a decrease in electric stability of

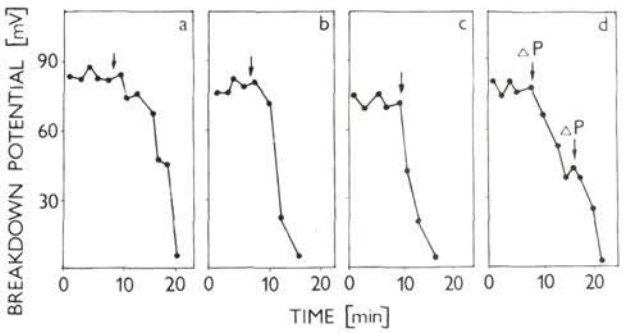

Fig. 2. The decrease of BLM electric stability following UV-irradiation (*a*), snake venom phospholipase A_2 (0.2 μg/ml) (*b*), protamine sulphate (15 μg/ml) (*c*), membrane expansion due to hydrostatic pressure difference between both sides of the membrane (3×10^{-3} N/m²) (*d*). *a*, *b*, *c* — BLM prepared from mitochondrial lipids dissolved in *n*-heptane (40 mg/ml) the bathing solution contained 50 mmol/l KCl, 1 mmol/l Tris-HCl, pH 7.4, the temperature was 39 °C. *d* — BLM formed egg lecithin in *n*-heptane (25 mg/ml), the bathing solution was the same.

bilayers (decrease in BLM electric breakdown potential). Possible mechanisms of the decrease in the electric stability will be considered in Discussion.

It is attractive to suppose that not only in the case of the BLM but also in biological membranes, these four fundamental factors acting in pathology may reduce the electrical stability of the lipid bilayer.

It has been shown in our experiments that ionic diffusion potential created across the membrane can break BLM no less efficiently than that applied from an external source (Puchkova et al. 1979).

An object which, in many respects, is a better model of cellular and intracellular vesicular membrane structures than planar BLM, is a phospholipid vesicle, the liposome. In particular, liposomal membranes do not contain organic solvent like heptane presented in BLM. Prior to starting measurements of the electrical breakdown potential in vesicular structures under membrane diffusional potential two problems must be considered: (1) which method should be used to create a controlled diffusion potential across the vesicular membrane, and (2) which are the criteria defining the loss of the membrane barrier function (a rapid increase in permeability).

To charge a liposome membrane different permeant ions may be used. A suitable method is to add a permeant acid from the outside of the vesicules and, after a proton gradient is created, to introduce a proton carrier into the suspension; this would result in the appearance of proton diffusion potential across the membrane (Puchkova et al. 1981). To detect the moment of the breakdown the light scattering method was used; it allowed to follow the osmotic behaviour of liposome. An increase or decrease in turbidity corresponds to vesicle shrinkage or

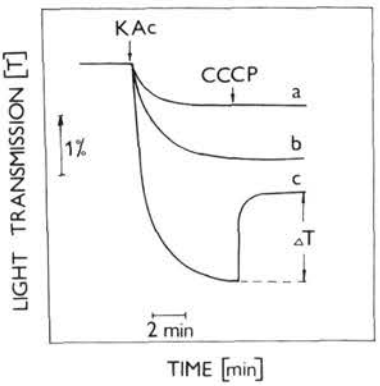

Fig. 3. Changes in light transmittance of liposomal suspension on the addition of CCCP (100 nmol/l). Liposomes (0.2 mg/ml) were formed from egg lecithin in sucrose solution (10 mmol/l) with KAc added: *a* — 5 mmol/l, *b* — 15 mmol/l, *c* — 40 mmol/l.

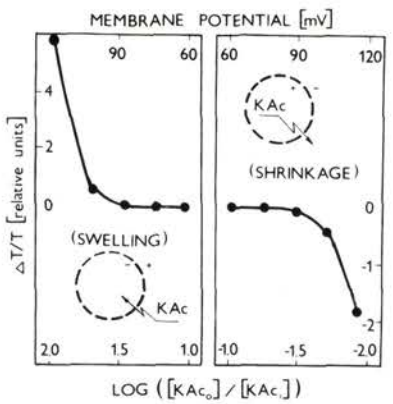

Fig. 4. Changes in light transmittance of liposomal suspension on the addition of CCCP to the liposomes loaded by KAc (*left*) or placed into KAc (*right*). Abscissa: *top* — logarithm of the KAc_o/KAc_i ratio (KAc concentrations outside — inside the liposomes); *bottom* — the calculated membrane potential. Ordinate — changes in membrane transmittance $\left(\frac{\Delta T}{T}\right)$ in response to the addition of CCCP. For other conditions, see legend to Fig. 3.

swelling, respectively. Fig. 3 illustrates electrical breakdown of a liposome membrane following the creation of a diffusion proton potential using hypertonic solution of potassium acetate (KAc). On KAc addition shrinkage of liposomes occurs and the turbidity increases. At the same time a pH gradient is created on the membrane as a result of the diffusion into the vesicles of nondissociated acetic acid (Singer and Bangham 1971). The addition of a proton carrier, CCCP (carbonyl-

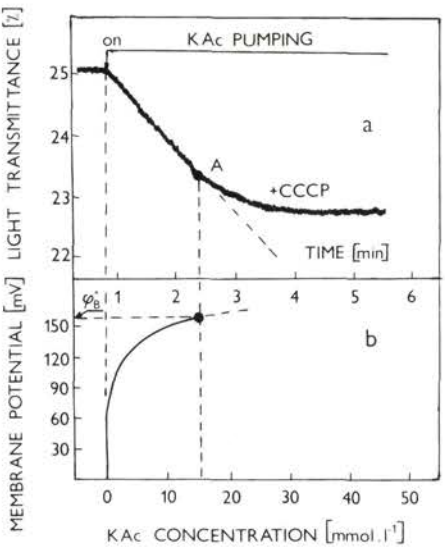

Fig. 5. Time-course of light transmittance (T) in the liposomal suspension (a) and the calculated membrane potential (b) during continuous introduction of concentrated potassium acetate solution. Liposomes (0.2 mg lipids/ml) prepared from egg lecithin were formed in 10 mmol/l sucrose solution. Final concentration of CCCP was 0.1 mmol/l.

cyanid-*m*-chlorophenylhydrosone) (Le Blank 1971), produces a membrane potential difference proportional to the transmembrane pH difference of solutions at both sides of the membrane. The potential difference in equilibrium will be:

$$\Delta\varphi = \frac{RT}{2F}(pH_0 + \lg C_0),$$

where C_0 is the KAc concentration in the medium; pH_0 is the pH value of the environment; R is the gas constant; T is absolute temperature and F is the Faraday constant.

It is clear from Fig. 3 that after liposome shrinkage had occurred the addition of protonophore had no effect on the vesicle volume at comparatively low KAc concentrations (and hence the membrane potential). However at KAc concentrations exeeding a certain critical value the addition of CCCP resulted in a sharp change in suspension turbidity, which may be attributed to a dramatic increase in membrane permeability. Apparently, at this concentration of KAc the membrane potential reached the critical value φ^* (Fig. 4) resulting in membrane electric breakdown. In another series of experiments liposomes at the state of formation were loaded with KAc in desirable concentrations. Then, the vesicles were placed into a sucrose medium containing no KAc (Fig. 4). Again, the addition of CCCP

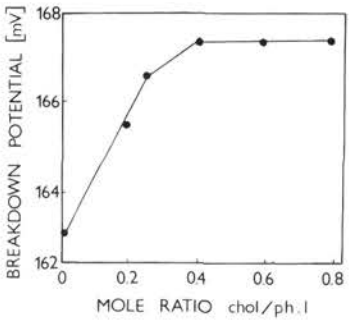

Fig. 6. Effect of cholesterol on electrical stability of the liposomal membranes. Liposomes (0.2 mg lipids/ml) were prepared from a mixture of rat liver mitochondrial phospholipids with cholesterol. For other conditions, see legend to Fig. 5.

at high concentrations of KAc led to dramatic changes in turbidity. The turbidity changes, however, were of the opposite direction as compared to the former case. The critical concentration of KAc seemed to be approximately the same regardless of whether KAc was inside or outside the vesicles.

Using the same approach we succeded in observing electric breakdown of liposomal membranes in suspensions containing NaAc + CCCP or ammonium sulphate + CCCP. The liposome behaviour in all these cases was uniform: at increasing salt concentrations up to some critical value the membrane barrier function was maintained and, then, a sudden increase in membrane permeability occurred, manifesting itself in gradual liposome swelling.

It is noteworthy that the curve in Fig. 4 resembles the voltage-current dependence curve for BLM at electric breakdown. To enable rapid estimation of breakdown potentials in liposomal suspension, a more convenient and faster modification of the method could be developed. In principle, a hypertonic solution of KAc is continuously added to liposome suspension. This results in monotonous shrinkage of liposomes and increased turbidity of the suspension (Fig. 5*a*). The monotony of the curve is broken in the presence of CCCP, when the membrane potential increases with the increasing KAc concentration in the solution (Fig. 5*b*). At a certain critical salt concentration the vesicle shrinkage becomes decelerated (point A). This point on the curve may be considered as the moment of electric breakdown. The concentration of KAc at this point may be used to calculate the breakdown potential according to equation shown above. This method may be employed only if no buffer is present inside the vesicles (since only in this case a sufficiently high ΔpH can be generated by the diffusion of a permeant acid), and if the membranes are impermeable to other ions (e.g. K^+) contained in the medium. For instance, the addition of valinomycine prevented the membrane electric breakdown to occur.

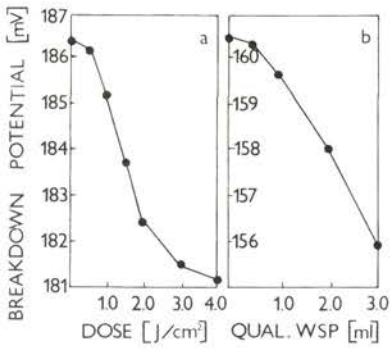

Fig. 7. Decrease in membrane electric stability of egg lecithin liposomes following UV-irradiation (*a*) and upon the addition of water-soluble products (WSP) of lipoperoxidation (*b*). WSP were isolated by centrifugation ($100{,}000 \times g$) of multilayer liposomes exposed to UV light (250—350 nm). WSP in supernatant showed an absorption maximum at 270—275 nm, had a heavy smell, and lowered the supernatant pH to 4.0—4.05. For other conditions, see Fig. 5.

It is well known that the structure and properties of the membrane lipid bilayer can be significantly modified in many ways, e.g. by introducting cholesterol, by the addition of surface active substances, by peroxidation of membrane lipid etc. In the present study, effect of cholesterol, some detergents and lipid peroxidation products on the breakdown potential were studied. The electrical stability of BLM is known to increase on the addition of cholesterol (Mikaelyan and Adzian 1978). A similar situation was observed in experiments with liposomes (Fig. 6). The value of the critical concentration of KAc in parallel with the increase in the bilayer microviscosity increased with increasing cholesterol content (Sagenin et al. 1982). The effects of cholesterol on both the viscosity and breakdown potential became much less pronounced at the cholesterol/phospholipid molar ratio above 0.4—0.5.

It has been shown earlier that the electric stability of BLM decreased under UV-induced lipid peroxidation in the membrane (Putvinsky and Puchkova 1981). A similar effect was also observed with egg lecithin liposomes (Fig. 7*a*). The electrical stability of lipsomes may be assumed to decrease as a result of accumulation of water-soluble LPO products which may influence the interface surface energy and increase the surface charge density on membrane (Deev et al. 1976). LPO products added to nonirradiated suspension also decreased the electrical stability of the membrane (Fig. 7*b*). The introduction of any of the three detergents used (negatively charged sodium dodecylsulphate (SDS), positively charged cetyltrimethylammonium bromide (CTAB), and a non-ionic(neutral) detergent, Triton X-100) produced a decrease in electric stability (Fig. 8). Apparently, it was a decrease in the membrane-water interface surface energy (σ) which was the cause of the drop of the membrane breakdown potential. Calcula-

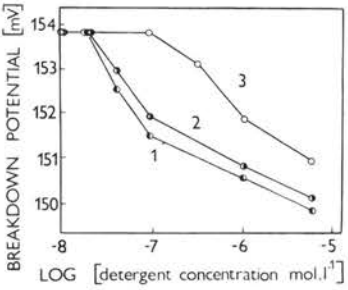

Fig. 8. Effects of detergents in membrane breakdown potential of liposomes prepared from egg lecithin. Liposomes were formed in sucrose solution containing different concentrations of detergents. 1 — sodium dodecylsulphate (SDS), 2 — cetyltrimethylammonium bromide (CTAB), 3 — Triton X-100. For other conditions, see Fig. 5.

tions based on the equation (Abidor et al. 1979) have shown that a decrease in breakdown potential by 3% of the initial value (which can be easily deteced by the method used in the present study) corresponds to a decrease in σ by only about 6%.

Discussion

Electrical stability seems to be one of the most important characteristic of the lipid bilayer. A decrease in stability may result in electrical breakdown of the membrane by the intrinsic potential of the membrane, and it may be an important mechanism by which membranes lose their barrier function in pathologic situation. In this respect, it is noteworthy that the four known causes of disturbances in the membrane barrier function in pathologic conditions (lipid peroxidation, phospholipase action, mechanical expansion of membrane, and changes in protein-lipid interaction) all result in a decrease in the lipid bilayer electrical stability (i.e. they markedly decrease the critical potential). However, it cannot be ruled out that the decreased barrier function may result from the appearance in membranes of more or less selective inducers of ion permeability, resembling ionophore antibiotics.

To answer the question whether the observed increase in membrane ionic conductivity is due to the action of an ionophore, or to electrical breakdown, the potential dependence of the effect should be measured. In a simple case, the inducers may increase membrane conductivity by the same factor at all potentials at which the membrane remains stable. The current-voltage plot for the membrane would be linear and the slope would increase. For BLM this is the case with water-soluble products of lipid peroxidation, which induce selective proton conductance of the phospholipid bilayer (Putvinsky et al. 1977; Truchmanova et al. 1976; Putvinsky and Puchkova 1981). The breakdown is shown as a break in the

voltage-current plot at the critical potential. Agents which decrease electric stability of the membrane shift the breakpoint to lower potentials.

The critical potential, i.e. the maximal potential difference at which the membrane has a certain electrical conductance and is mechanical stable, may serve as the quantitative characteristic of the membrane electrical stability. At potentials higher than critical, electrical breakdown of membranes occurs. We could show that the values of critical potentials for BLM and liposomes lay within the range of 100—200 mV, i.e. they are comparable with potentials existing in the living cells. Model experiments performed in this paper have shown that even a small decrease in the critical potential as a result of lipid peroxidation, phospholipase action, membrane osmotic expansion and protein adsorption may result in electrical breakdown of the membrane by the potentials, which in the living cell are generated by the membrane itself.

The electrical breakdown theory developed in the laboratory of Prof. Chizmadzhev (Abidor et al. 1978; Abidor et al. 1979) provides the understanding of the mechanism underlying decreases in electrical stability in pathologic conditions. Three parameters have the key position in the generation of breakdown potential: surface tension on the membrane-water interface, mechanical expansion of the membrane, and asymmetrical surface potential.

According to the theory, a decay of the surface tension should lead to a decrease of the breakdown potential. In our experiments all the detergents decreased the breakdown potential (Fig. 8).

LPO products as well as products of phospholipid hydrolysis by phospholipase A_2 are surface-active agents. Due to this they can decrease electrical stability of the membrane. Mechanical expansion influences surface energy and the critical potential similarly as do detergents. Finally, the asymmetrical surface potential must also play an important role. It is known that breakdown develops at a certain value of potential drop, φ, on the lipid bilayer, which in turn is result of superposition of the transmembrane potential, φ_m, and the difference between surface potentials, φ_s (the inside minus outside potentials).

LPO products (as well as those of phospholipase action) have negative charges (Deev et al. 1976) and they decrease φ^* like other negatively charged detergents, such as SDS. On the other hand, quite different compounds, polypeptides and proteins, when adsorbed on the lipid bilayer, can also influence the electrical stability. Apparently they may act both as surface-active agents and by changing the surface potential.

The occurrence of electrical breakdown in model lipid membranes produced by ion diffusion potentials, the relatively low critical potentials of membrane stability (comparable to the potentials existing on membranes of living cells), a dramatic decrease of the critical potential value (for lipid bilayer) by four main mechanisms by which membranes can lose their barrier function (lipid perox-

idation, phospholipase action, osmotic expansion, and protein adsorption), all support the hypothesis that electrical breakdown significantly contributes to the damage to biological membranes and disturbances of cell functioning in pathologic conditions.

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