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AN INVESTIGATION OF ELECTRICAL BREAKDOWN OF BIMOLECULAR LIPID MEMBRANES

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The investigation is concerned with the irreversible electrical breakdown of bimolecular lipid membranes, depending on the velocity of linear voltage scanning. It was found that the membrane breakdown potential depended on the velocity of electric field variation. For instance, at voltage scanning velocities of up to 0.1 V/s, the rupture of membrane from glycerol monooleate occurs at 0.20–0.25 V and, at velocities higher than 1 V/s, at 0.5–0.6 V. Then the film breakdown depending on lipid phase transition was studied. At high velocities of imposed voltage scanning, the disruption of the bimolecular lipid membranes was shown not to depend on their phase states; at the same time, at low velocities, one could note a slight difference in the stability of the films at temperatures higher and lower than those of the phase transition. Whereas transition from gel to liquid-crystalline state involves transition from an ordered to a less ordered membrane structure with a sharp increase in the number of defects in the membrane, the authors, conclude that the film breakdown in the second case occurs by the 'defect' mechanism suggested earlier. It was also assumed that, in certain cases involving low velocities of voltage scanning, membrane breakdown may occur because of variation in the interfacial tension and in the contact angle between the film and torus. Possible mechanisms of the membrane irreversible electrical breakdown at high velocities of voltage variation are discussed. It was shown that breakdown should occur as a result of membrane compression in an electric field by a mechanism previously examined. The elastic moduli of a number of membranes were calculated by the breakdown criterion suggested earlier. They were found to coincide with the results of other investigators and, depending on the type of lipid, to equal 10^5–10^6 Pa.

Introduction

Two theories have been presented to explain the irreversible electrical breakdown of bimolecular lipid membranes. Crowley's mechanism [1] is based on bimolecular lipid membrane compression in an electric field. In this case, Crowley represents the bilayer in the form of a homogeneous elastic film, to which Hook's equation is applicable. In Crowley's view, membrane breakdown occurs after the electric field had attained a certain pressure exceeding the bimolecular lipid membrane elastic force, a pressure at which irreversible deformation develops in the bilayer. Abidor et al. [2,3] regard the bimolecular lipid membrane breakdown as due to structural disorders (defects) in the membrane matrix. In their view, bimolecular lipid membrane rupture in the electric field occurs as a result of their increase to critical values.

Reconstitution of defects in the electric field results in the migration of lipid molecules in the bimolecular lipid membrane plane. The lateral diffusion coefficient (D) of phospholipids in lipid membranes is about 10^{12} m^2/s [4,5]. According to other evaluations [6], the defect may form over an area of up to 1000 Å^2. Since the lipid molecule in the bilayer occupies an area of 50–75 Å^2 [7], as many as 15 molecules may be involved in defect formation. Einstein's equation $t=(15L)^2/D$ may be applied for evaluating the time (t) required for displacing the defect to a distance of (L) 8Å, which corresponds to the pore diameter in the bimolecular lipid membrane [8]. As one can see, $t \approx 0.1$ ms, wherefrom it is apparent that the process of defect reconstruction is quite slow. At high velocities of electric field variations in bimolecular lipid membrane, one can foresee a situation in which the defects would not keep pace with the field variations. Since structural disorders in the bilayer would, in this case, not be tangible, the membrane will behave as the homogeneous elastic film examined by Crowley [1], and the bimolecular lipid membrane breakdown mechanism should vary accordingly.

The present work examines the mechanisms of the electrical breakdown of lipid membranes from various phospholipids at linear scanning of transmembrane potential. To clarify the role of defects in bimolecular lipid membrane disruption in an electric field, we studied the membrane breakdown at lipid phase transition from gel to liquid-crystalline state.

Materials and Methods

Bimolecular lipid membranes were formed by the method of Muller et al. in the aperture (diameter 1 mm) located in the Teflon partition. Two chambers separated by the partition were filled with aqueous solutions of either 0.001 mol/dm^3 potassium chloride (chemically pure) or 0.001 mol/dm^3 Tris-HCl (Reanal) and 0.001 mol/dm^3 citric acid (chemically pure). In the latter case, the solution pH equalled 6.5 ± 0.1. The cell was thermostatically controlled.

The membranes were formed from glycerol monooleate (Serva), dipalmitoylphosphatidylethanolamine (DPPE) (Koch-Light), dimyristoylphosphatidylcholine (DMPC) (Serdary Research Laboratory), and egg lecithin (Koch-Light). Experiments with egg lecithin were duplicated with a lipid kindly provided by Dr. E.Ya. Kostetsky. In the latter case the lipid fatty acid composition included 49% residue of palmitic acid, 11% stearic acid, 36% oleic acid, and 3.9% linoleic acid. Other fatty acids were detected in trace amounts.

Lipid purity was checked by thin-layer chromatography. Glycerol monooleate and DPPE were used without additional purification. DMPC was purified by thin-layer chromatography until a single spot was obtained. Each forming lipid solution contained 20 mg/ml of the substance. Glycerol monooleate was dissolved in *n*-decane (chromatographically pure) and egg lecithin in *n*-dodecane (chemically pure); the DPPE and DMPC solutions were prepared on a solvent mixture consisting of *n*-dodecane, chloroform (chemically pure) and methanol (chemically pure) (7:2:1, v/v). Membranes from glycerol monooleate were formed at 20 ± 0.5°C, from egg lecithin and DMPC at 26 ± 0.5°C, and from DPPE at 66 ± 0.5°C.

Membrane formation was controlled visually in an MBS-2 microscope and also by variations in capacitance. Capacitance was measured at 0.2 Hz at linearly varying voltage, amplitude 50 mV. The installation was composed of a special-form generator (G6-15), an electrometer (VA-J-51, G.D.R.) and an oscilloscope (C1-19). Capacitance were measured by means of silver-silver chloride electrodes (MB-1M3). Their asymmetry potential did not exceed 1 mV. The measurement technique is described in greater detail elsewhere [9].

Membrane breakdown was established by the circuit current. Up to potential scanning velocities of 0.5 V/s inclusive, a block of polarising voltages of polarograph PPT-1 was used. Higher velocities of potential variations were obtained when the membrane capacitance was charged at generating potential pulses of varying amplitudes from a G5-46 pulse generator. The film breakdown times were determined by means of a C8-1 storage oscilloscope by a sharp rise in the circuit current following bimolecular lipid membrane breakdown. Platinum grid electrodes were used for breaking down the membranes.

Results and Discussions

Fig. 1 shows the breakdown potentials of membrane from glycerol monooleate in an electric field depending on the logarithm of voltage variation velocities over bimolecular lipid membrane. At low potential scanning velocities of up to 0.04 V/s,

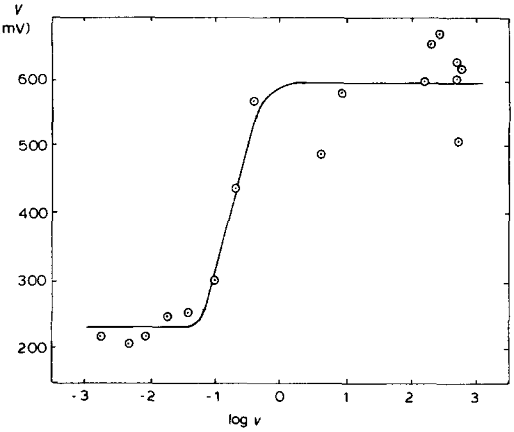

Fig. 1. Dependence of membrane potential breakdown upon the logarithm of voltage scanning velocity. Bimolecular lipid membranes were formed from an glycerol monooleate (20 mg/ml) in *n*-decane. The aqueous solution contained 0.001 mol/dm^3 potassium chloride. Experimental temperature 20.0 ± 0.5°C. Each points represents the mean, which was obtained from 10–30 measurements. For log v (abscissa), v is measured in V/s.

bimolecular lipid membrane broke down at 200–250 mV. This corresponds to the breakdown voltage of the majority of membranes from other lipids [10]. Increase in voltage scanning velocity to values higher than 0.04 V/s led to an ultimate 2-fold rise in the breakdown potential. At scanning equal to 1 V/s, bimolecular lipid membrane breakdown occurred at 500–600 mV, no longer depending on voltage variation velocity. A similar picture was observed on bilayers from other lipids. For some membranes studied in the present work, the breakdown potentials corresponding to low and high voltage scanning velocities are shown in Table I.

It must be remarked that our experimental results are concerned with irreversible electrical breakdown of bimolecular lipid membrane under prolonged (up to 0.5 ms) application of potential difference. In a recent communication [11] it was reported that a reversible high conductance state occurs with charging times in the range between 10 ns and 10000 ns. This state was defined as an reversible electrical breakdown. This reversible breakdown of lipid membranes occurs at much higher constant potential than the irreversible rupture of bimolecular lipid membrane.

The dependence of bilayer breakdown on electric field variation velocities is indicative of different bimolecular lipid membrane breakdown possibilities, and the presence of two plateaux on the curve in Fig. 1 permits there being a minimum of two such possibilities and that membrane breakdown in both cases occurs in quasi-equilibrium conditions. Correspondingly, each possibility must have its respective bimolecular lipid membrane breakdown mechanism.

Another work [12] is also indicative of membrane electrical breakdown by various mechanisms; it studied the dependence of the capacitance of bilayers from diisostearoylphosphatidylcholine upon transmembrane potential difference upon application of sinusoidal alternating current and showed that at frequencies below 1 Hz electrical breakdown occurs at 120 mV, and at 60 Hz and more at 200 mV. Since the bimolecular lipid membrane electrical characteristics depended on a.c. frequency, the author assumed that the breakdown mechanisms at low and high frequencies may vary.

The question is, what membrane breakdown mechanisms could take place in the electric field? Abidor et al. [2] showed that, with electrical breakdown through defects, the film lifetime is in inverse proportional dependence upon the number of defects in their structure. By somehow changing their number, one can determine the conditions in which bimolecular lipid membrane breakdown occurs on account of disorders in the membrane structure. For instance, one can examine in an electric field the lifetime of membrane existing in different phase states. With transition from gel to liquid-crystalline state, denser molecule packing in the bilayer is replaced by a less dense packing to lead to growing disordering of the hydrocarbon chains and to a resultant increasing number of defects in the bimolecular lipid membrane structure [13,14]. Hence, phase transition would change the membrane lifetime in the electric field. Thus, it would be possible to show what potential scanning velocities the bimolecular lipid membrane breakdown corresponds to by the 'defect' mechanism.

To that end, we measured the lifetimes of membranes from glycerol monooleate at 10–25°C. A corresponding diagram is shown in Fig. 2, where curve 1 was obtained at potential scanning equal

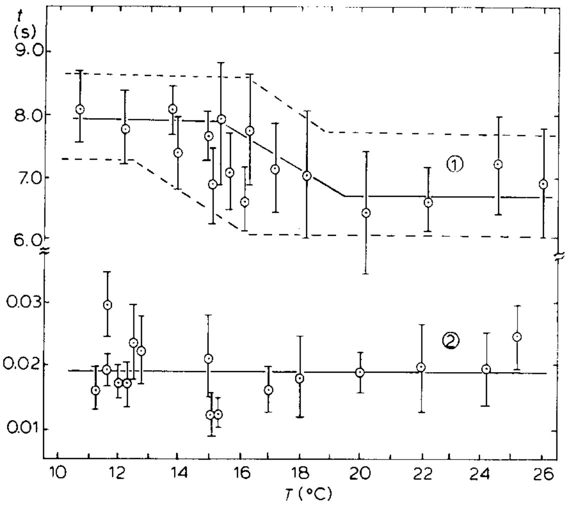

Fig. 2. Dependence of lifetime of membranes from glycerol monooleate upon temperature at voltage scanning velocities equal to 0.04 V/s (1) and 250 V/s (2). Each point represents the mean with the standard deviation which was obtained from 10–25 measurements.

to 0.04 V/s, and curve 2 at 250 V/s. If our assumption that bimolecular lipid membrane breakdown in electric field depends on defects at phase transition is true, one could expect a heat-generating break to appear at 15–17°C, the temperature at which glycerol monooleate melts in the bilayers [15,16]. As is apparent from Fig. 2, at high voltage variation velocities corresponding to the upper plateau in Fig. 1, the lifetimes of membranes in the electric field do not depend on temperature, and no breaks are observed in curve 2. Hence, membrane phase state does not affect electrical breakdown. At low potential scanning velocities (curve 1) corresponding to the lower plateau in Fig. 1, the membrane lifetimes in the electric field at temperatures lower and higher than those at phase transition are somewhat different. The transition range is 14–20°C, i.e. in the field of phase transformation. Although temperature dependence is not clearly pronounced, the effect of phase transformation on membrane breakdown at low voltage scanning velocities cannot be completely ruled out. Hence, it should be recognized that bilayer structural disorders could be the cause of their breakdown in the electric field.

The results of experiments for studying the temperature dependencies of membrane lifetimes in an electric field once again indicate the possibility of bimolecular lipid membrane breakdown by various mechanisms. If one were to accept with certain qualifications that bimolecular lipid membrane breakdown at low potential scanning velocities involves defects, than the question arises concerning mechanism of electrical breakdown at high voltage variation velocities. In this connection, it would be appropriate to examine the investigation results of electric field effects on bimolecular lipid membrane.

Examined experimental data for bimolecular lipid membrane capacitance dependence upon transmembrane potential difference are indicative of variations in bimolecular lipid membrane area and thickness [17–22]. Increase in bilayer area in an electric field is caused by increase in the contact angle between the bimolecular lipid membrane and the torus, and also between the bimolecular lipid membrane and the surface lens, this being in turn caused by a decline in the film interfacial tension [23,24]. As was shown in [25], stable membranes are formed at a definite contact angle between the film and torus. Consequently, variations of the contact angle in the electric field may destabilize the bimolecular lipid membrane and ostensibly cause breakdown.

Decrease in bimolecular lipid membrane thickness and increase in bimolecular lipid membrane area at application of transmembrane potential difference occurs in parallel. Any of the two factors may be the cause of bimolecular lipid membrane electrical breakdown, but it is not clear which. A study of bimolecular lipid membrane reflectivity in transmitted polarized light [19,26] showed that upon application of the electric field, the bimolecular lipid membrane thickness decreases in the initial moment, and the bimolecular lipid membrane area subsequently increases. Relative variation in membrane thickness amounts to 0.02–0.04% (at 100 mV). The time constant of the process depends on the lipid and solvent used. For example, for membranes from egg lecithin dissolved in *n*-heptane, it 0.10–0.15 ms, and for the same membranes from *n*-decane 1–2 ms [26]. The time constant of contact angle variation is considerably higher. A study of the behaviour of the lens on the surface of a membrane from xylane-0 showed [27] that the variation frequency of its shape corresponds to the current frequency to as much as 1 Hz. With the current frequency higher than 10 Hz, the contact angle cannot keep up with variations in the electric field; it simply increases by the same magnitude as at application of the transmembrane potential difference constant, which equals the alternating current amplitude. The contact angle variation time constant was not estimated, but it apparently constitutes splits of a second.

An examination of the investigation results for the effect of an electric field on bimolecular lipid membrane shows that, at high potential scanning velocities corresponding to the upper plateau in Fig. 1, membrane breakdown should occur because of compression; i.e., it should occur by the mechanism suggested by Crowley [1]. In our view, at low velocities of electrical field variation, one can also not exclude the possibility of bimolecular lipid membrane breakdown on account of contact angle variations. If not in all cases, this could anyhow occasionally take place when the defects in the membrane structure are relatively few. One could expect that membrane breakdown caused by variations in area would occur on bimolecular lipid membrane from glycerol monooleate; for these bilayers, the dependence of their lifetime in an electric field does not change too much with more frequent structural disorders at bimolecular lipid membrane phase transitions (curve 1, Fig. 2).

In examining the possible mechanisms of electrical breakdown of bimolecular lipid membranes, one conclusion important for subsequent argumentation has been made. It was shown that, irrespective of the mechanism by which the bimolecular lipid membranes are broken down at low potential scanning velocities (as a result of defects in their structure or due to contact angle variations), bimolecular lipid membrane breakdown at high velocities occurs as a result of compression. This permits to use the Crowley criterion [1] for assessing membrane breakdown in an electric field. According to Crowley [1], the breakdown critical potential (V_{cr}) determines the forces of bimolecular lipid membrane electrical compression, these forces being equal to bimolecular lipid membrane elastic forces. An only slight increase in field voltage violates this equilibrium and causes irreversible deformation and breakdown of the bilayer. Crowley expresses his criterion for membrane electrical breakdown as follows:

$$\frac{C_0 V_{cr}^2}{2Eh} \approx 0.18,$$

where C_0 is the bimolecular lipid membrane capacitance, h its thickness, and E the Young modulus. At high voltage scanning velocities, when one may assume that electrical breakdown occurs as a result of decrease in bimolecular lipid membrane thickness, let us use this criterion and calculate the elastic moduli for a number of membranes that we studied (see Table I).

From Table I, it is apparent that, depending on the type of lipid used, the Young modulus in a direction perpendicular to the bimolecular lipid membrane plane is equal to 10^5–10^6 Pa. According to Crowley [1], who used underestimated values of bimolecular lipid membrane breakdown potentials, E equalled 10^4–10^5 Pa. Hence, the values of his elastic moduli are one order of magnitude lower than those found by us and other authors. For instance, for membranes from egg lecithin, $E \approx 3 \cdot 10^5$ Pa [29] and $5 \cdot 10^5$ Pa [30],

TABLE I

CAPACITANCE, THICKNESSES AND BREAKDOWN POTENTIALS AT LOW (V_l) AND HIGH (V_h) VOLTAGE SCANNING VELOCITIES, AND ALSO ELASTIC MODULI OF SOME BIMOLECULAR LIPID MEMBRANES

Lipid	C_0 (μF/cm^2)	h (Å)	V_l (V)	V_h (V)	E (Pa)($\times 10^{-5}$)
Glycerol monooleate	0.41 [16]	44.9 [16]	0.2–0.25	0.55	7.7
DPPE	0.4	50	0.2	0.45	4.5
DMPC	0.3	50	0.15	0.26	1.1
Egg lecithin	0.339 [28]	54.8 [28]	0.15–0.2	0.32	1.8

while in Crowley's work [1] $E \approx 2.9 \cdot 10^4$ Pa. Our result is $1.8 \cdot 10^5$ Pa.

From the above-cited comparison of Young's moduli, calculated in the present work and determined by other investigators, it follows that the method of electrical breakdown may be used for estimating these moduli. Compared with other techniques, the principal advantage of this method lies in simplicity. Further studies in this direction could be concentrated on revealing and testifying bimolecular lipid membrane breakdown mechnisms in an electric field. After that, the method of electrical breakdown could find wider application in studing the viscoelastic properties of model and biological membranes.

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