Kinetics of pore size during irreversible electrical breakdown of lipid bilayer membranes

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ABSTRACT The kinetics of pore formation followed by mechanical rupture of lipid bilayer membranes were investigated in detail by using the charge-pulse method. Membranes of various compositions were charged to a sufficiently high voltage to induce mechanical breakdown. The subsequent decrease of membrane voltage was used to calculate the conductance. During mechanical breakdown, which was probably caused by the widening of one single pore, the membrane conductance was a linear and not exponential function of time after the initial starting process. In a large number of experiments using various lipids and electrolytes, the characteristic opening process of the pore turned out to be independent of the actual membrane potential and electrolyte concentration.

Our theoretical analysis of the pore formation suggested that the voltage-induced irreversible breakdown is due to a decrease in edge energy when the pore had formed. After initiation of the pore, the electrical contribution to surface tension is negligible. The time course of the increase of pore size shows that our model of the irreversible breakdown is in good agreement with mechanical properties of membranes reported elsewhere.

INTRODUCTION

External electrical fields are widely used for manipulation of artificial and biological membranes (for a review, see Zimmermann, 1982; Zimmermann et al., 1981, 1989). Electroinjection of macromolecules into living cells and electrofusion of cells (without detoriation of cellular and membrane functions) are used today in many laboratories for genetic engineering and somatic hybridization (Zimmermann et al., 1989, 1991). In contrast to chemically and mechanically mediated pertubation of the membrane (Taupin et al., 1975; Lucy and Ahkong, 1986; Evans and Needham, 1987), application of electric field pulses of sufficient field strength and short duration has the advantage of being predictable.

Despite the widespread use of these field pulse techniques in biology, medicine, and biotechnology (Zimmermann et al., 1991), the underlying mechanism of the reversible breakdown of the membrane is not yet satisfactorily understood. In addition, our knowledge about the mechanisms which lead to a transition from a reversible to an irreversible electrical breakdown of a biological membrane is very poor. However, this information is required to improve the present field pulse techniques for application such as human hybridoma technology and in other fields of industrial interest.

Most theories on the underlying mechanism of irreversible breakdown were derived on the basis of experimental data obtained from artificial lipid bilayer membranes. The reason for this is obvious. In contrast to biological membranes, the lateral homogeneity of such black lipid membranes considerably facilitates the experimental approach and the theoretical analysis of the data. As shown by Benz and Zimmermann (Benz et al., 1979; Benz and Zimmermann, 1980, 1981), these structures exhibit both reversible and irreversible electrical breakdown of the membrane, depending on the field strength and duration of the applied pulses. The similarity of the phenomena, therefore, allows some conclusions about the possible mechanisms of the corresponding field effects in biological membranes.

Careful and detailed studies have been performed on the lifetime of the bilayer membrane under the action of an external electrical field (for a review, see Chernomordik et al., 1987). Other groups have shown the role of membrane tension on the breakdown (Needham and Hochmuth, 1989). However, the breakdown process itself has not been analyzed so far, although it is crucial for the validity of any theoretical consideration. The question whether one or several pores lead to mechanical (and irreversible) breakdown or the time course of the widening of the pore(s) have not yet been investigated. The only information is that mechanical breakdown takes place in the subsecond time range. In this work, we therefore measured in lipid bilayer membranes of various composition the kinetics of the diameter increase of the pore, which finally leads to the mechanical rupture of the lipid membranes. Our data suggest that this process is controlled by macroscopic mechanical forces. Furthermore, it is evident that the viscosity of the membrane plays an important role in the increase of the pore diameter.

THEORETICAL BACKGROUND

Several groups have considered the lifetime of lipid bilayer membranes under high electric fields (Barnett and Weaver, 1991; Chernomordik et al., 1987). The common starting point of all those models is that the stability of a membrane is pertubed by defects, which are described by two opposite energy contributions

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$$E_{\text{defect}} = 2\pi a\gamma - \pi a^2\sigma. \quad (1)$$

The first term represents the energy needed to build up the edge of a pore of radius a where γ stands for the line tension. The second term is the energy, which is gained by the increase of the size of the pore due to the surface tension σ. Pores may be formed either by thermal activation or by mechanical pertubation. If the radius a of the pore is below the critical one $a^* = \gamma/\sigma$, the edge energy dominates and the pore tends to close. In the opposite case the surface tension leads to an increase of the pore size, which results in irreversible breakdown of the membrane.

In the past (quoted in Barnett and Weaver, 1991; Chernomordik et al., 1987) the effect of a transverse electric field has been introduced into Eq. 1 by adding the condenser energy of the lipid bilayer. Inside the pore the lipid was thought to be replaced with nonconducting water. Under this assumption the electric potential does not alter after a defect comes into existence, and the electric field acts simply as an additional surface tension.

However, if the extreme difference in conductivity between the membrane and the ionic solution ($\sim$10 orders of magnitudes) is taken into account, the electric field distribution will be changed even by small defects. It can be shown that such effects cause an electrical contribution to both the line tension and the surface tension (Winterhalter and Helfrich, 1987). The electrical line tension $\gamma_{\text{el}} = -\epsilon_w\epsilon_0 U_0^2$ tries to open the pore, and the negative contribution to the surface tension $\sigma_{\text{el}} = -\epsilon_l\epsilon_0 U_0^2/2d$ emanating from the field along the membrane surface tends to close it again. Recalling these earlier results for a defect in an externally imposed transverse potential, we obtain

$$E_{\text{defect}} = 2\pi a\gamma_{\text{eff}} - \pi a^2\sigma_{\text{eff}} = 2\pi a(\gamma - \epsilon_w\epsilon_0 U_0^2/2\pi) - \pi a^2(\sigma - \epsilon_l\epsilon_0 U_0^2/2d). \quad (2)$$

Here d is the membrane thickness and U_0 the applied voltage, whereas $\epsilon_w = 80$, $\epsilon_l = 2$ are the relative permittivity of water and of lipid, and $\epsilon_0 = 8.8 \cdot 10^{-12}$ As/Vm is the permittivity of vacuum. Although we did not alter the formal character of Eq. 1 by replacing the two mechanical parameters γ and σ by their effective counterparts, we now obtain two different possible mechanisms. Using the applied potential U_0 as parameter and increasing it continuously, Eq. 2 shows that either first $\gamma_{\text{eff}} = 0$ or $\sigma_{\text{eff}} = 0$. In the first case an enhancement of the field reduces the critical radius and promotes rupture. In the second case an increase of U_0 stabilizes the membrane and causes stable pores at higher voltages. As the mechanical parameters of the lipid under investigation favor the first process, we refer for further discussion of the second process to our earlier paper (Winterhalter and Helfrich, 1987).

If thermal fluctuation is neglected, then Eq. 2 allows an irreversible breakdown only if the radius a becomes larger than the critical one $a > a^* = \gamma_{\text{eff}}/\sigma_{\text{eff}}$. Inserting the range of values, $\gamma = 1\text{–}2 \cdot 10^{-11}$ N, obtained for similar lipids by Chernomordik et al. (1987) requires breakdown voltages of about $U_0 \approx 400$ mV for γ_{eff} to vanish. Inspection of Eq. 2 shows that the applied voltage (400 mV) together with a bilayer thickness $d = 5$ nm is responsible only for a relatively small electrical contribution to the surface tension $\sigma_{\text{el}} = 0.3 \cdot 10^{-3}$ N/m in comparison with the mechanical one of roughly $2\text{–}3 \cdot 10^{-3}$ N/m. We may therefore neglect the electrical part and take $\sigma_{\text{eff}} \approx \sigma$ to be independent of the applied voltage with good accuracy. After the initial step of pore forming, the opening process is driven by the mechanical surface tension. Simultaneously, the conductivity of the pores causes the membrane to discharge. If the remaining potential keeps γ_{eff} small enough, the opening process becomes independent of the applied voltage. Once the pore becomes appreciably larger than the critical mechanical radius $a^* = \gamma/\sigma$, such edge effects do not influence the further opening.

To understand our experimental data (see below), we have to model the dynamic behavior of a pore, which leads to an irreversible breakdown. Due to the finite surface tension, the lipid is homogeneously stretched like a soap film. Furthermore, we assume that the pore is of cylindrical shape and large enough to neglect effects due to a finite membrane thickness. During the opening process, lipid starts to flow radially away from the center to the torus of the membrane. Unfortunately, an exact solution of the membrane material flow requires the knowledge of the local stress distribution after breakdown. Whatever the local stress distribution will look like, the surface tension will be the driving force for the opening. In a first approach one would expect that the membrane viscosity will determine the time course of the opening. However, as shown in Appendix A, this will be lead to an exponential increase of the pore radii with time, which is in contrast to our finding. In this context we like to recall the results by Frankel and Mysels (1969). They reinvestigated the bursting of soap films. After initiating the breakdown with a spark, they recorded the kinetics of the opening with a high-speed camera. As a first approximation they supposed that the film itself stays immobile and only the lipid material of the hole will move with constant velocity. The change in elastic energy due to the pore formation is balanced by the increase in kinetic energy due to the constant velocity of the lipid material of the pore in form of a precurser film. The kinetics will be obtained by including

$$E_c = \tfrac{1}{2}\pi a^2 \rho d(\dot{a})^2, \quad (3)$$

into Eq. 2. Neglecting the electrostatic contribution to the surface tension as well as the edge effect, we obtain (Appendix A) the time dependency of the pore radii

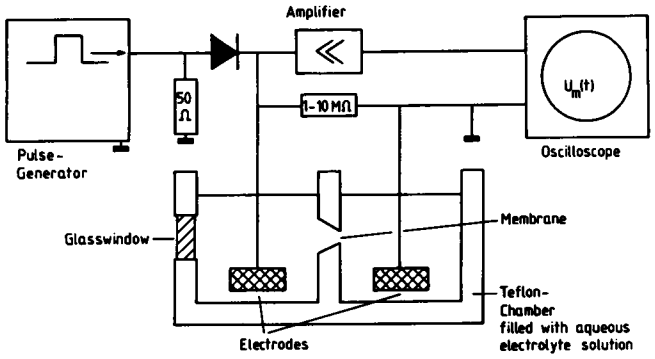

FIGURE 1 Scheme of the charge-pulse instrumentation used for the measurement of electric field-induced irreversible breakdown of lipid bilayer membranes.

$$a(t) = a_0 + \sqrt{\frac{\Phi\sigma}{d\rho}}\, t = a_0 + \alpha t, \qquad (4)$$

with Φ as a parameter taking irreversible contributions into account.

In a third step we relate these theoretical results to the measured time course of the resistance. Using the underlying potential distribution (Appendix B), the resistance of a defect with radius a is given by:

$$R_{\text{defect}} = \frac{1}{2\kappa a}. \qquad (5)$$

Here κ stands for the conductivity of the aqueous solution. Inserting $a(t)$ in Eq. 5 yields the following relation for the conductivity $G(t)$ versus time:

$$G(t) = \frac{1}{R_{\text{defect}}} = 2\kappa(a_0 + \alpha t) \qquad (6)$$

MATERIALS AND METHODS

Optically, black lipid bilayer membranes were made in the usual way (Mueller et al., 1963; Benz and Läuger, 1976) from a 1% solution of lipid in *n*-decane (purum; Fluka, Buchs, Switzerland). The following lipids were used for formation of membranes: diphytanoyl phosphatidylcholine, dioleyl phosphatidylethanolamine, phosphatidylserine, and azolectin (all from Avanti Polar Lipids, Birmingham, AL). The membrane cell was made of Teflon. The circular holes in the wall between the two compartments had areas between 0.3 and 3 mm^2. The unbuffered aqueous salt solutions (analytical grade; Merck, Darmstadt, Germany) were prepared with double distilled water and had a pH of ~6. The temperature was kept at 20°C.

The membrane was charged through extremely fast Ag/AgCl platinum black electrodes (Annex Instruments, Santa Ana, CA) in series with a diode (reverse resistance > 10^{12} Ω, 2N 5653; Pan, Taufkirchen, Germany) by short current pulses of 0.2–10 μs duration generated by a commercial pulse generator (PM 5770; Philips, Sweden), having a rise-time better than 10 ns (Fig. 1). The actual membrane voltage was measured with an operational amplifier (gain 10–20-fold, input resistance 10^{12} Ω, bandwidth 10 MHz). The amplified signal was fed into a digital oscilloscope (model 4094; Nicolet Instruments Corp., Madison, WI) with 8-bit resolution of the 16 k words of minimal 20 ns per point.

The digital signal was analyzed with a computer (model 9820 AD; Hewlett-Packard Palo Alto, CA). The value of the membrane capacitance was calculated from the RC-time constant of the exponential discharge process of the membrane voltage (initial voltage 20–50 mV) across an external resistor (1–10 MΩ; Fig. 1) in parallel to the membrane. During irreversible breakdown, the actual membrane conductance, $G(t)$, i.e. the conductance of the pore(s) or other defect structure(s), were calculated according the following equation:

$$G(t) = I(t)/U(t) = \frac{1}{U(t)}\frac{\partial Q(t)}{\partial t} = \frac{1}{U(t)}\frac{\partial(CU(t))}{\partial t} = \frac{C}{U(t)}\frac{\partial U(t)}{\partial t}. \qquad (7)$$

$U(t)$ was the actual membrane voltage. $G(t)$ was calculated under the assumption that the membrane capacitance, C, was independent of time. Because of the limited amplitude resolution of the digital oscilloscope, the voltage signal had a considerable scatter, which did not allow the precise calculation of $G(t)$. Therefore, $U(t)$ was obtained by averaging nine successive values for $U(t)$ (4 before t and 4 after t, all with a time interval of 20 ns) to obtain a smooth curve for the time course of the membrane conductance, $G(t)$.

RESULTS

Irreversible breakdown of lipid bilayer membranes

After formation of the membrane, the value of the capacitance was calculated from the RC-time constant of the discharge process of the membrane capacitance, C, across an external resistor, R (typically 1 or 10 MΩ). The membrane was charged to a small initial voltages in the range between 20 and 50 mV to avoid mechanical pertubations. The specific capacities (i.e., the capacitance per unit area) of the membranes made of different lipids were very similar to those reported previously (Benz and Janko, 1976). Afterward the membranes were charged to higher initial voltages on the order of 300–800 mV. It is interesting to note that such membrane potentials are sufficient to cause reversible electrical breakdown on oxidized cholesterol membranes (Benz and Zimmermann, 1980) but not on the membrane systems used in the present investigation. As a consequence of the high electric field, the membranes broke mechanically during the discharge process, when the initial voltage was above 400 mV. Mechanical breakdown was indicated by a rapid discharge process. However, we could not find a clear correlation between the initial voltage and the start of the breakdown process.

Fig. 2 shows typical time courses of these discharge processes measured on four different membranes made of diphytanoyl phosphatidylcholine/n-decane and bathed in a 1 M KCl-solution. The membranes were charged by a 1-μs-long current pulse to initial voltages between 480 and 530 mV. After the end of the charge pulse, the voltage showed some minor decay, which was probably caused by a small increase of the membrane capacitance and had nothing to do with the time resolu-

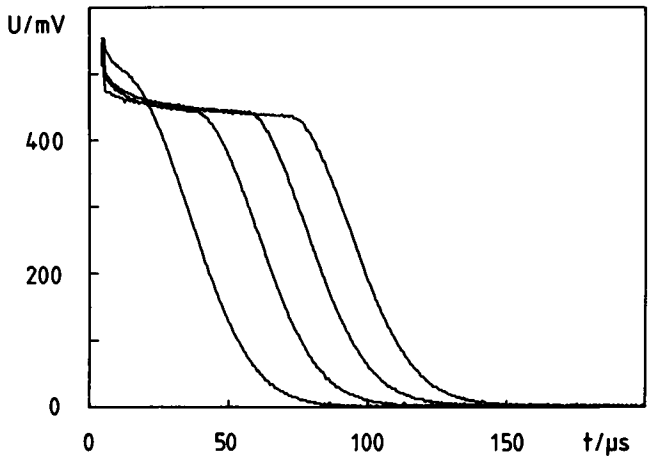

FIGURE 2 Time course of membrane voltage $U(t)$ during electric field-induced irreversible breakdown of four membranes formed of diphytanoyl phosphatidylcholine/n-decane. The pulse length of the charge pulse was 1 μs. The aqueous phase contained 1 M KCl; T = 20°C.

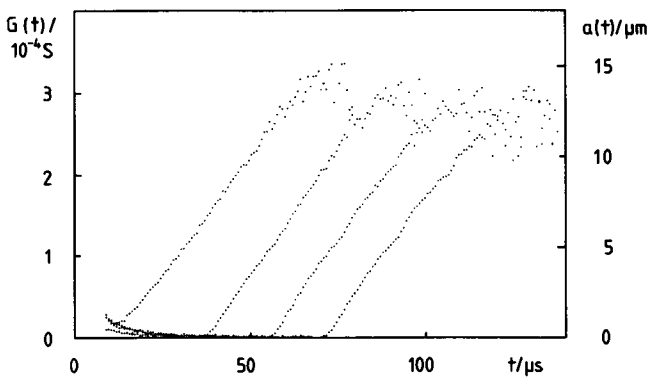

FIGURE 3 Conductance $G(t)$ versus time curves during irreversible breakdown of the four different diphytanoyl phosphatidylcholine membranes of Fig. 3. $G(t)$ was calculated according to Eq. 8 by using the capacitance of the individual membranes. The conductance immediately after the end of the charge pulse represents capacitance relaxations due to the high electric field. The radii of the pores are indicated at the right-hand ordinate.

tion of the detecting system. This capacitance increase was caused by Maxwells pressure as a result of the high electric field. It reflected the thinning of the membranes and/or the lateral shift of the solvent in so called "microlenses" (Benz and Janko, 1976; Alvarez and Latorre, 1978). Without breakdown the membrane discharged in a slow exponential decay due to the shunt resistor. Pores appeared randomly and were seen as suddenly as the membrane potential dropped to zero within ~50 μs (Fig. 2). The four different curves of Fig. 2 represented different breakdown experiments under approximately the same starting conditions but the begin of the breakdown varied between 10 and 80 μs.

Analysis of the breakdown experiments

The plots of voltage versus time of breakdown experiments similar to those shown in Fig. 2 were analyzed using the formalism given in Materials and Methods. Fig. 3 represents the analysis of the four $U(t)$ curves of Fig. 2 by using Eq. 6. The conductance increase during irreversible breakdown of all four membranes was a linear function of time for ≥50 μs. Furthermore, the slope of the conductance versus time curves was approximately the same for all four curves. It has to be noted that such linear conductance-time relationships were observed for all lipids investigated in this study.

One or several pores during irreversible breakdown?

In a recent publication it has been suggested that reversible and irreversible breakdown occur uniformly in a lipid bilayer membrane (Barnett and Weaver, 1991). Such a behavior has in fact been observed for the reversible breakdown of lipid bilayer membranes (Benz et al., 1979). The result presented here suggested on the other hand that only one or a small number of pores were involved in mechanical rupture, otherwise we should have observed a considerable variation of the curve slopes of Fig. 3. Careful inspection of the slopes of a large number of experiments revealed a few experiments, in which a twofold change of the slope was observed after the start of the breakdown process. Fig. 4 shows the conductance-time curve of such an experiment. The initial slope of the curve was 0.3 S/s. After ~20 μs the slope changed to 0.6 S/s, which was approximately twice the

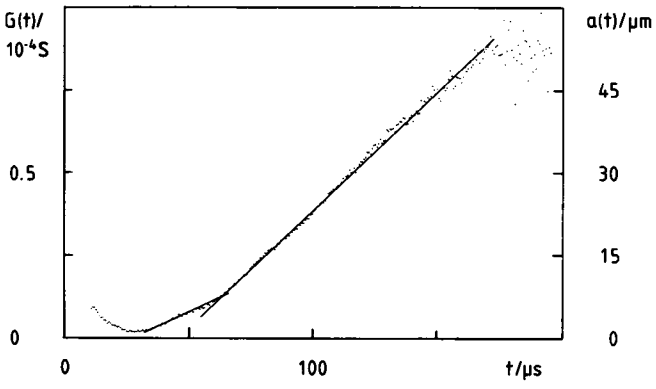

FIGURE 4 Conductance $G(t)$ versus time curve during irreversible breakdown of a diphytanoyl phosphatidylcholine membranes bathed in 100 mM LiCl. $G(t)$ was calculated according to Eq. 8 by using the capacitance of the individual membranes. The duration of the charge pulse was 1 μs; T = 20°C. Note that the initial slope of the curve was first 0.3 S/s and changed after about 10 μs to 0.6 S/s, presumably due to the opening of a second pore. The radius of the pore are indicated at the right-hand ordinate.

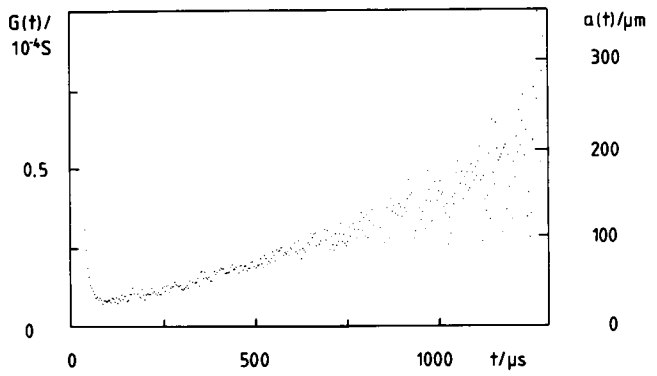

FIGURE 5 Conductance $G(t)$ versus time curve during irreversible breakdown of a diphytanoyl phosphatidylcholine membranes bathed in 10 mM KCl. $G(t)$ was calculated according to Eq. 8 by using the individual capacitance of the membrane. The duration of the charge pulse was 1 μs; T = 20°C. At the end of the considered time interval (1 ms), the area of the pore was approximately one tenth of the total membrane area. The radius of the pore is indicated at the right-hand ordinate.

mean value from all experiments performed under the same conditions. This result was consistent with the assumption that field-induced mechanical breakdown of membranes occurs by the formation and increase in size of one single pore.

Influence of ionic strength

After correction for the specific conductivity of the bulk aqueous phase, κ, the ionic strength of the aqueous solutions had little if any influence on the slope. Fig. 5 shows the conductance versus time plot of a breakdown experiment, which was performed in 10 mM KCl instead of 1 M KCl. Again the curve was linear, but the slope was much smaller, which may be understood on the basis of the smaller specific conductivity of the bulk aqueous phase at 10 mM KCl (1.4 instead of 112 mS/cm). The interesting outcome of the experiments at small ionic strength was that the conductance-time curve could be followed for a much longer time. Whereas at a concentration of 1 M KCl the linear range could only be followed for ~50 μs, Fig. 5 demonstrates that it was at least as long as 1 ms at an aqueous salt concentration of 10 mM. This result suggested that it was linear for even longer times.

Size of the pore during irreversible electrical breakdown

As pointed out in Theoretical Considerations and also in Appendix B, the field across a membrane pore is not constant when the pore radius is much greater than its length. For $a \gg d$, the resistance of the pore is given by its access resistance (Hille, 1984) (Appendix B). Eq. B4 was used for the calculation of the pore size from the actual conductance $G(t)$ at a given time. The radii of the pores are indicated at the right-hand ordinates of Figs. 3–5. Whereas the small ionic strength (10 mM) of the experiment of Fig. 5 allowed the pore size to be followed up to a radius of ~200 μm, it could only be observed up to 10 μm in the case of 1 M KCl.

Increase of pore radius with time during mechanical rupture

The calculation of the pore size from the conductance data allowed the estimation of the increase of the radius with time. The change of the pore radius with time obtained for different systems gave a more meaningful comparison for the parameters varied in our study. For each of the five lipids used here, two series of experiments were performed. In the first series we used KCl as electrolyte at four different concentrations (10, 100, 1,000, and 3,000 mM). In a second set of experimentals, KCl was replaced by the less conductive LiCl to find out whether different ions influence the velocity of the radii increase. The results of these experiments are summarized in Tables 1 and 2. No significant difference was found for the different conditions.

Each experiment used for the calculation of the data of Tables 1 and 2 was repeated between 5 and 40 times. Furthermore, we also varied pulse length, initial voltage, and membrane capacitance to find out if these parameters had any influence on the velocity of the conductance increase. The experimental results for one set of conditions are given in Table 3 (dioleoyl phosphatidylcholine, 1 M KCl, 20°C). The delay time is the interval between a charge pulse and the start of mechanical rupture. Table 3 shows none of these parameters had influence on the slope within the limits of accuracy. Only a few extremes were found. Some of them were presumably caused by the formation of more than one pore during breakdown.

TABLE 1 Slope of the pore radius increase during irreversible breakdown for different lipids and KCl-concentrations

	10 mM KCl	100 mM KCl	1 M KCl	3 M KCl
Conductivity, S/m	0.14	1.4	11.2	28
		α [m/s]		
Dioleyl-PC	0.22 ± 0.1	0.25 ± 0.1	0.34 ± 0.2	0.84 ± 0.3
Dioleyl-PE	0.93 ± 0.1	0.78 ± 0.1	1.25 ± 0.2	0.84 ± 0.3
Diph-PC	0.17 ± 0.02	0.39 ± 0.1	0.45 ± 0.2	0.49 ± 0.03
Asolectin	0.62 ± 0.2	0.43 ± 0.2	0.35 ± 0.2	0.43 ± 0.2
PS	0.50 ± 0.1	0.47 ± 0.2	0.40 ± 0.2	0.50 ± 0.2

The membranes were formed from 1% (wt/vol) solutions of the different lipids in n-decane. The current-pulse for the charging of the membranes was between 0.5 and 10 μs. The initial voltage was between 350 and 500 mV. α was calculated from the slope of the individual conductance-time curves according to Eqs. 6 and 7 from ≥10 membranes by using the corresponding bulk aqueous conductivities κ. T = 20°C.

TABLE 2 **Slope of the pore radius increase during irreversible breakdown for different lipids and LiCl-concentrations**

	10 mM LiCl	100 mM LiCl	1 M LiCl	3 M LiCl
Conductivity, S/m	0.09	0.84	6.1	12.6
		α [m/s]		
Dioleyl-PC	0.43 ± 0.2	0.20 ± 0.03	0.42 ± 0.1	0.38 ± 0.1
Dioleyl-PE	0.50 ± 0.2	0.57 ± 0.1	0.74 ± 0.1	0.70 ± 0.04
Diph-PC	0.33 ± 0.1	0.40 ± 0.05	0.50 ± 0.1	0.47 ± 0.1
Asolectin	0.08 ± 0.02	0.24 ± 0.04	0.2 ± 0.03	0.19 ± 0.04
PS	0.28 ± 0.02	0.23 ± 0.01	0.63 ± 0.05	0.78 ± 0.1

The membranes were formed from 1% (wt/vol) solutions of the different lipids in n-decane. The current-pulse for the charging of the membranes was between 0.5 and 10 μs. The initial voltage was between 350 and 500 mV. α was calculated from the slope of the individual conductance-time curves according to Eqs. 6 and 7 from ≥10 membranes by using the corresponding bulk aqueous conductivities, κ. T = 20°C.

In one case we observed very small slope, which had no apparent explanation. In any case, the data of Table 3 clearly demonstrate that the size of the membrane had no influence on the slope of the conductance increase.

DISCUSSION

In this study we measured the conductance increase of a pore, which leads to mechanical rupture of a lipid bilayer membrane. The irreversible breakdown of the membranes was induced by a high electric field pulse. The pore conductance was obtained from the time dependence of the membrane potential by using the charge-pulse method. This method is characterized by very good time resolution (Benz and Läuger, 1976; Läuger et al., 1981; Benz, 1988) as well as only imposing a very small current through the electrodes. Without the latter characteristic, it would have been very difficult to measure the pore conductance with sufficient accuracy because of diffusion polarization effects at the electrodes.

One of the basic problems of the interpretation of the experiments reported here is the assumption of a time independence of the membrane capacitance during the breakdown process. In fact, the charge-pulse experiments performed at very high initial voltage suggest some capacitance relaxation as indicated by Fig. 2. On the other hand, it has been demonstrated that the real compressibility of lipid bilayer membranes is very small (Alvarez and Latorre, 1978) and that the electric field-induced lateral shift of solvent into microlenses is a slow process (Benz and Janko, 1976). The surface area of the pore responsible for breakdown is at maximum (at 10 mM KCl, i.e., under the conditions of Fig. 5) 10% of the total membrane area. This means that the membrane capacitance changed only slightly before and during irreversible breakdown.

We are convinced that the field-induced rupture of a lipid bilayer membrane has the same kinetics as the normal process, which has a lifetime that is statistically distributed. Pores within lipid bilayer membranes likely come into existence by overcoming the energy barrier (Chernomordik et al., 1987), and the probability for this process is described by a Boltzmann distribution, $\exp(E_{defect}/kT)$. This probability is very low in the absence of mechanical pertubations, and lipid bilayer membranes thus have a long lifetime in the absence of membrane potentials. When an external field is applied, the probability for the formation of pores is increased. Nevertheless, it appears to be a random process since the pores open with widely scattered delays (~0.5–50 μs) after the end of the pulse. This observation and the fact that the slope of the conductance versus time curve is very similar for most of the pores show clearly that only one or a few pores were generated. Theories based on the assumption of the formation of a large number of small pores spread uniformly across the whole bilayer during irreversible electrical breakdown (Barnett and Weaver, 1991) would produce a large scatter of individual breakdown onsets and of the conductance versus time slopes. This means that those theories are obviously in contradiction to our experimental findings.

TABLE 3 **Slopes of the conductance versus time curve during irreversible breakdown of 26 different diphytanoyl phosphatidylcholine membranes in 1 M KCl**

Capacity	Pulse-length	Voltage ϕ_0	Delay-time	Slope
nF	*μs*	*V*	*μs*	*S/s*
3.1	0.5	0.536	24.4	4.26
3.1	0.5	0.502	0	38
3.1	0.75	0.452	0	46
3.1	0.75	0.293	19.5	18.2
3.1	0.75	0.695	0	45.5
3.1	0.75	0.578	0	8.8
3.1	1	0.611	2	29.4
3.1	1	0.435	2	8
3.1	1	0.427	129	7.6
3.1	2	0.494	41	10.3
3.1	4	0.511	56	9.8
3.1	7	0.544	2	8.2
3.1	15	0.318	34	9.4
0.12	0.2	0.524	14	7.84
0.12	0.5	0.703	1.7	13.2
0.12	0.75	0.729	0	13.4
0.12	1	0.578	56	1.97
3.8	0.2	0.511	12	10.2
3.8	0.2	0.441	0	10.2
3.0	0.75	0.447	44	9.1
3.2	1	0.423	7.3	9.2
3.6	2	0.488	73	8
3.5	4	0.476	4.8	9.5
3.6	4	0.441	41	10.5
3.7	7.2	0.452	61	8.6
3.8	10	0.502	46	8.8

The membranes were formed from 1% (wt/vol) solutions of the lipid in n-decane. The slope of the curves were calculated from plots similar to those given in Fig. 3; T = 20°C.

The membrane potential has only a small influence (if any) on the conductance increase during mechanical breakdown once the pore has been formed. Thus, the role of the electric field is only to decrease the energy barrier for pore formation. Higher field strengths increase the probability for the formation of pores, but they do not influence the kinetics of pore opening. If one or more pores have been formed, then their further fate is only controlled by mechanical parameters of the membrane such as surface tension or viscosity. These results seem to contradict other theories of mechanical breakdown (Barnett and Weaver, 1991) in which the electric field acts like an additional tension even after the pore is formed. This contradiction may be explained by assuming that the electrical field collapses in and near the breakdown pore because of the high conductivity of the aqueous salt solution. As a consequence the electric field-induced surface tension vanishes.

The experimental data presented here clearly show a linear increase of conductance with time. This has not been observed up to date. We related our data to an earlier model by Winterhalter and Helfrich (1987) on the origin of electrical breakdown. As an additional and independent support of this model, we checked whether the experimentally measured parameters are in agreement with other mechanical properties of membranes (Hochmuth and Evans, 1987; Waugh, 1987). We found that the surface tension that drives the opening of the pore is damped not only by surface viscosity but also by inertia. Viscosity would cause an exponential increase instead of the observed linear one. It is interesting to note that our observation is in agreement with a similar problem in soap films (Frankel and Mysels, 1969). Careful inspection of the curve shown in Fig. 5 at longer times shows that the conductance-time relationship was slightly superlinear. This may be caused by viscosity effects, but this has to be investigated more carefully in the future.

The results presented here provide some evidence that the model proposed by Winterhalter and Helfrich (1987) is correct. This model proposes that electrical breakdown of membranes also may occur due to a decrease in edge energy. Such a conclusion may have some relevance to the fusion of lipids (without electrical fields) with detergents or with polyethyleneglycol. This assumes that other effects such as thickness changes do not contribute and overcompensate these effects (Dimitrov and Jain, 1984). Either an increase of the surface tension and/or a decrease of the edge energy will promote fusion.

APPENDIX A

According to the discussion outlined in Theoretical Background, we take into account that around the critical pore radii the mechanical edge energy cancels with the electrical one and that at later times for large pores such edge effects becomes negligible. In addition, we pointed out that in our experiments the electrical contribution to the surface tension are very small and will be disregarded. The dynamical problem reduces itself to a pure mechanical one.

In the first part we like to review an earlier calculation by Frankel and Mysels (1969) to point out that a linear increase of the opening velocity $\partial a/\partial t = \dot{a}$ of the pore is within their model. They assume a homogeneous stress σ (in their calculation twice the interfacial stress) within the membrane that falls as a step function to 0 at the edge of the pore. This force is

$$f_a^{\sigma} = 2\pi a\sigma, \tag{A1}$$

and is to be counterbalanced by the change in momentum of the excess membrane material of the relaxed part which is supposed to be found in a tiny rim around the pore. Furthermore, it is assumed that the pore opening velocity $\dot{a} = const.$ The change in momentum yields the force at the edge

$$f_a^{\text{kinetic}} = -\frac{\partial}{\partial t}(\pi a^2 \rho d)\dot{a} = 2\pi a\dot{a}^2\rho d. \tag{A2}$$

A simple solution for $\dot{a}$ is given by

$$\dot{a} = \sqrt{\frac{\sigma}{d\rho}}. \tag{A3}$$

In a more refined model we allow that the unstressed membrane material to be distributed instead in an infinitely thin rim at the edge but in a finite one. Again we assume that the stress is homogeneous and no membrane material is moving beyond a given radii, but R is replaced by a where the latter corresponds to the edge. Incompressibility of the lipid requires the local radial material velocity $v(r)$ to be related to the radial flow at R

$$v(r) = \frac{R}{r}\dot{R}. \tag{A4}$$

An integration over the local momentum distribution give finally a similar force as Eq. A2 but where a, $\dot{a}$ are to be replaced by R and $\dot{R}$. The relation between this two radii is estimated within the Hook'schen approximation via $\sigma = k_E(\Delta A/A) = k_E(\pi a^2/\pi R^2)$ (Evans and Needham, 1987). This finally yields an equation for the pore velocity accounting for a finite rim

$$\dot{a} = \sqrt{\frac{\sigma}{k_E}}\sqrt{\frac{\sigma}{d\rho}} = \sqrt{\frac{\Phi\sigma}{d\rho}}, \tag{A5}$$

where in Φ we lumped the effects of the unknown flow profile as well as viscosity effects in.

In the second part we show that including the lipid viscosity in a straight way yields an exponential increase of the pore size. Starting again with the driving force of the opening given by Eq. A1 but neglecting inertia terms. This radial flow will be damped by the small but finite viscosity of the lipid which causes a tension $\sigma_\eta(r) = 2\eta[\partial v(r)/\partial r] = -2\eta(a\dot{a}/r^2)$, where $\eta \approx 1 \cdot 10^{-9}$ Ns/m (Waugh, 1987; personal communication) stands for the surface viscosity. This inhomogeneous tension causes at the edge of the pore a force

$$f_a^{\eta} = -8\pi\eta\dot{a}, \tag{A6}$$

which has to be balanced by the stress term of Eq. A1. Obviously this leads for the pore size an exponential increase

$$a = a_0 e^{(\sigma/4\eta)t}. \tag{A7}$$

Inserting reasonable values for $\eta \approx 10^{-9}$ Ns/m and $\sigma \approx 2 \cdot 10^{-3}$ N/m yields for the decay time $\tau = 4\eta/\sigma \approx 2 \cdot 10^{-6}$ s. As in our measurements, we recorded the conductivity over several orders of magnitude

longer than τ any exponential increase would be clearly visible. In this context we like to mention an earlier report (Sukharev et al., 1982) using voltage clamp on a BLM in 1 M KCl solution. Within the time range of a few microseconds after the initiation of the breakdown an exponential increase has been measured that can be attributed to viscosity.

APPENDIX B

A first approximation for the potential distribution of a flat membrane with a circular defect of radius a in the field of a plate condenser was proposed some time ago (Winterhalter and Helfrich, 1987):

$$U(r, z) = \pm \frac{U_0}{\pi} \times \operatorname{arccot}\left(\frac{2a^2}{r^2 + z^2 - a^2 + [(r^2 + z^2 - a^2)^2 + 4a^2z^2]^{1/2}} \right)^{1/2}, \quad \text{(B1)}$$

where $r^2 = x^2 + y^2$ and U_0 are the potential difference across the membrane far away from the pore. The positive sign hold for the upper side ($z > 0$) of the membrane and negative for the lower side that coincides with the xy-plane. The electric field distribution emanating from this potential ansatz is equivalent to that of an infinitely thin cylindrical conductive plate carrying a total charge of $Q = 4U_0\epsilon_w\epsilon_0 a$. If we assume a linear relation between the current density i and the electric field strength E

$$\mathbf{i} = \kappa \mathbf{E} \quad \text{(Ohm's law)}, \quad \text{(B2)}$$

where κ represents the specific conductivity. It is easy to obtain the resistance of the entire pore via the total current I through it:

$$I = \int_0^a \kappa E_z dx dy = \frac{\kappa}{\epsilon_w \epsilon_0} \frac{Q}{2}. \quad \text{(B3)}$$

The resistance is obtained by Ohm's law

$$R_{\text{defect}} = \frac{U_0}{I} = \frac{1}{2\kappa a}. \quad \text{(B4)}$$

This resistance is exactly the same as what is sometimes termed the access resistor (Hille, 1984). The underlying approximation is that the membrane thickness is negligable in relation to pore radius. Inspection of the measured values of conductivity in our systems shows that this is always fulfilled.

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