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PULSE-LENGTH DEPENDENCE OF THE ELECTRICAL BREAKDOWN IN LIPID BILAYER MEMBRANES

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Summary

Charge-pulse experiments were performed on artificial lipid bilayer membranes with charging times in the range between 10 ns and 10 μs. If the membranes are charged to voltages in the order of 100 mV, the membrane voltage at the end of the charge pulse is a linear function of the injected charge. However, if the membranes are charged to voltages in the range of 1 V, this relationship no longer holds and a reversible high conductance state occurs. This state is defined as an electrical breakdown and it does not allow the membranes to charge to higher voltages than the breakdown voltage, V_c. Between charging times of 300 ns and 5 μs at 25°C and between 100 ns and 2 μs at 40°C, V_c showed a strong dependence on the charging time of the membrane and decreased from 1.2 to 0.5 V (25°C) and from 1 to 0.4 V (40°C). For other charging times below and above these ranges, the breakdown voltage seemed to be constant. The results indicate that the breakdown phenomenon occurs in less than 10 ns.

The pulse-length dependence of the breakdown voltage is consistent with the interpretation of the electrical breakdown mechanism in terms of the electromechanical model. However, it seems possible that below a charging time of the membrane of 300 ns (25°C) and 100 ns (40°C) other processes (such as the Born energy) become possible.

Charge-pulse experiments on the giant algal cell, *Valonia utricularis*, have demonstrated that, when large amounts of charge are injected into the vacuole, the electrical resistance between the vacuole and medium falls dramatically. The voltage at this electrical breakdown varies with the time taken to charge the cell in the range from 0.8 to 10 μs [1]. At a charging time

of 0.8 μs the breakdown voltage (2.4 V) is about twice as large as the corresponding values at a pulse length of 10 μs (1.1 V) [1]. The interpretation of the pulse-length dependence of the breakdown voltage of cells of *V. utricularis* is not straightforward, since we are dealing with two membranes in series, i.e., the tonoplast and plasmalemma. Relaxation studies in the low-field range indicated that the observed pulse-length dependence of the breakdown voltage is probably not due to the different time constants of the two membranes, but reflects some intrinsic property of a single membrane. If this is caused by the lipid bilayer, artificial lipid bilayer membranes should exhibit a similar relationship between the charging time and the absolute value of the breakdown voltage. It has recently been demonstrated [2] that artificial lipid bilayers made from oxidized cholesterol show a breakdown phenomenon similar to that measured on cell membranes. The breakdown voltage of artificial lipid bilayers has been found to be of the order of 1 V at a charging time of 500 ns (25° C) [2]. This reversible electrical breakdown phenomenon has to be carefully distinguished from the mechanical (irreversible) breakdown which is observed at much lower voltages (for bilayers made up of oxidized cholesterol at approx. 350 mV).

In this communication we report on charge-pulse measurements in which the charging time was varied between 10 ns and 10 μs. For this purpose, the following set-up was used. A fast commercial pulse generator (Hewlett Packard 214 B) with a rise time of 10 ns and a maximum output voltage of 100 V at 50 Ω was connected to the membrane cell through a diode with a reverse voltage resistance of approx. $1 \cdot 10^9$ Ω. The actual voltage across the membrane was measured with a Tektronix 7633 (7A13 amplifier, 1 M Ω input resistance) storage oscilliscope (band-width 80 MHz). The set-up was tested carefully with dummy circuits as described earlier [1, 2]. The time resolution of the instrumentation was approx. 40 ns for an output voltage of the pulse generator of 10 V and 100—150 ns for higher voltages. Black lipid bilayers were obtained in the usual way [3] from a solution of oxidized cholesterol in *n*-decane (1—2%, w/v). The membranes were bathed in 1 M KCl and had a capacitance of approx. 10 nF (membrane area 2 mm^2, specific capacitance 555 $nF \cdot cm^{-2}$). The membranes were kept at 10 or 25° C throughout.

Charge pulses of constant length and increasing injected charge were applied to the membranes. Typical experiments are given in Figs. 1 and 2. In Fig. 1 four charge pulses of 10 ns duration but increasing injected charge ($8.3 \cdot 10^{-9}$ to $1.6 \cdot 10^{-8}$ C) were applied to the same membrane. After the end of the charge pulses, the diode in the charging circuit is reverse biased and thus the voltage across the membrane can only decay by charge movements across the membrane. For small charge pulses (not shown) the voltage across the membrane at the end of the pulses increases with the amount of charge injected according to the relationship, $V = Q/C$. However, for large pulses (Figs. 1 and 2) these potentials no longer increase, but even decrease. This phenomenon must mean that for sufficiently high voltages the membrane resistance decreases dramatically, thus allowing part of the injected charge to cross the membrane within the period of the pulse. In Fig. 1 this breakdown of the membrane resistance has occurred within 10 ns if, during the

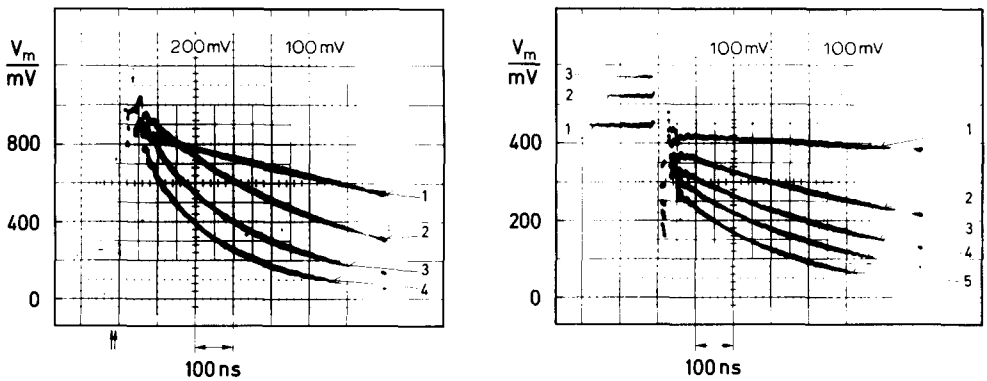

Fig. 1. Oscillographic record of charge-pulse experiments performed on a membrane made from oxidized cholesterol/*n*-decane at 25°C. Four charge pulses of 10 ns duration and increasing charge (trace 1: $8.3 \cdot 10^{-9}$ C to trace 4: $1.6 \cdot 10^{-8}$ C) were applied to the membrane (membrane capacitance 9.2 nF) bathed in 1 M KCl. The maximum voltage (V_c) across the membrane at the end of the charge pulses was estimated to be 1.2 V (dead-time of the measuring device approx. 100 ns). The arrows indicate the beginning and end of the four charge pulses.

Fig. 2. Oscillographic record of charge-pulse experiments performed on a membrane made from oxidized cholesterol/*n*-decane at 25°C. Five charge pulses of 5 μs duration and increasing charge (trace 1: $4.1 \cdot 10^{-9}$ C to trace 5 : $6.5 \cdot 10^{-9}$ C) were applied to the same membrane as in Fig. 1 (membrane capacitance 9.2 nF). The maximum voltage (V_c) across the membrane at the end of the charge pulse was estimated to be 450 mV (dead-time of the measuring device approx. 50 ns). The traces at the left-hand side of the figure show the end of the charge pulses of trace 1 to trace 3.

pulse, the voltage has exceeded 1.2 V. The highly conducting state is also reflected in the fast decays of the membrane voltage across the membrane at the end of the charge pulses. Trace 4 has a time constant for the exponential decay of approx. 200 ns. With the assumption that the specific capacitance of the membrane (555 nF $\cdot$ cm^{-2}) is not changed very much during breakdown, a specific resistance of 0.4 $\Omega \cdot$ cm^2 is calculated from the RC-time constant of the membrane. This means that the specific resistance of the membrane switches from $1 \cdot 10^8$ $\Omega \cdot$ cm^2, observed at low voltages, to less than 0.4 $\Omega \cdot$ cm^2 during breakdown. From breakdown experiments with a charging time of 10 ns it can be shown that the breakdown phenomenon is even faster than this short time.

Results of a breakdown experiment with a much longer charging time are presented in Fig. 2. Five charge pulses with a constant duration of 5 μs but increasing charge (trace 1:$4.1 \cdot 10^{-9}$ C to trace 5:$6.5 \cdot 10^{-9}$ C) were applied to the same membrane. As a result of the conducting process described above, the initial voltage across the membrane decreased for charge pulses with higher injected charge and the membrane could not be charged to a higher voltage than 450 mV. The different breakdown voltages observed for 10 ns and 5 μs pulse lengths indicate that the breakdown voltage is a function of the charging time. Further measurements of the breakdown voltage using charging times between 10 ns and 10 μs support this conclusion.

The pulse-length dependence of the breakdown voltage measured at two different temperatures (25 and 40°C) is given in Figs. 3 and 4. The data presented are average values from more than 20 independent experiments. It is evident that the breakdown voltage markedly increased below a pulse length of approx. 10 μs with decreasing charging time and assumed a constant value

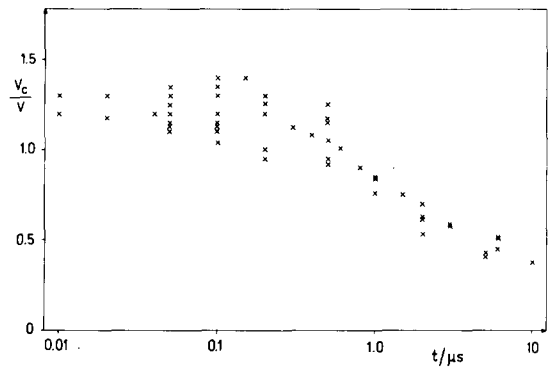

Fig. 3. Breakdown voltage, V_c, of lipid bilayer membranes made from oxidized cholesterol/*n*-decane as a function of the charging time. V_c is defined as the maximum voltage the membranes can be charged to at a given pulse length. T = 25°C, 1 M KCl. The results were taken from 15 different membranes.

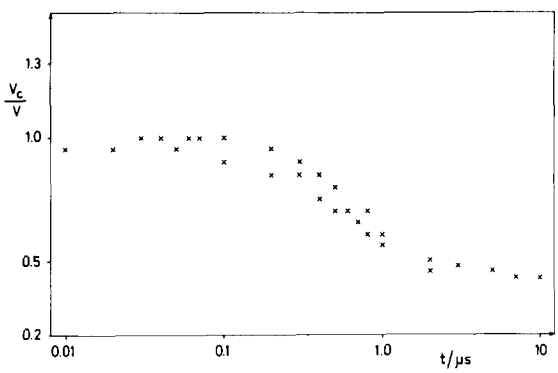

Fig. 4. Breakdown voltage, V_c, of lipid bilayer membranes made from oxidized cholesterol/*n*-decane as a function of the charging time. V_c is defined as the maximum voltage the membranes can be charged to at a given pulse length. T = 40°C, 1 M KCl. The results were taken from 12 different membranes.

below a pulse length of 800 ns. Whilst the breakdown voltage is of the order of 0.5 V at 10 μs charging time, it rises to approx. 1.2 V in the range 10--800 ns (25°C, Fig. 3). On the other hand, when the charging time exceeds 10 μs the breakdown voltage again seems to become independent of the pulse length. This is particularly confirmed by corresponding measurements at 40°C (Fig. 4). At this temperature we can prove beyond doubt that the breakdown voltage reaches a constant value of approx. 400 mV above a charging time of 10 μs. This value is slightly higher (by approx. 50 mV) than the critical voltage resulting in irreversible mechanical breakdown observed in the 100 μs range. The temperature dependence of the breakdown voltage in the pulse-length range 10—800 ns is in keeping with the results we reported recently [2] (2 V at 4°C and 1 V at 40°C). The pulse-length dependence of the breakdown voltage of artificial lipid bilayers is similar to that for cells of *V. utricularis*, with the exception that in this species we could not prove unequivocally that a plateau value for the breakdown voltage is reached towards very short charge pulses because the resolution of the set-up was too low in the *Valonia* experiments [1].

The finding reported here for bilayers supports the assumption that the pulse-length dependence of the breakdown voltage is an intrinsic property of a single membrane. Such a relationship is expected if the mechanism of breakdown is interpreted in terms of the electromechanical model as discussed by Zimmermann et al. [8] (see also for review, Ref. 4). In this model it is assumed that the membrane, or parts thereof, can be regarded as a capacitor filled with a homogeneous elastic dielectric material. It is postulated that the thickness of the membrane capacitor depends on the electrical compressive forces (arising from the membrane potential) and on the mechanical forces (arising either from the internal hydrostatic pressure, i.e., turgor pressure in walled cells or from externally applied pressure). It can be theoretically shown that, towards higher voltages or pressures, a critical thickness is reached at which the compressive forces are no longer counterbalanced by the elastic restoring forces resulting in a reversible breakdown. The quantitative treatment shows that the breakdown voltage depends on the elastic compressive modulus transverse to the membrane plane (Y_m), the relative dielectric constant (ϵ) and the unstressed membrane thickness (δ_0) at a pressure of $P = 0$:

$$V_c(P = 0) = \left(\frac{0.37 \cdot Y_m}{\epsilon_0 \cdot \epsilon}\right)^{1/2} \cdot \delta_0 \qquad (1)$$

The electromechanical model predicts a pressure dependence of the breakdown voltage ([9] see also Eqn. 4 in Ref. 4), a coupling between pressure in walled cells and membrane potential changes, which should result in some circumstances in the generation of action potentials and effects of high absolute (hydrostatic) pressures in a hyperbaric chamber on the membrane permeability. These predictions have been confirmed very recently indicating that the fundamental ideas of this model, at least, are correct [4]. The pulse-length dependence of the breakdown voltage is easily explained if we assume that the membrane material is not perfectly elastic and shows inertia. In this case, Y_m should become a function of the compression time of the membrane and increase with increasing rate of compression, as is shown for other viscoelastic materials [4]. According to Eqn. 1 an increase in Y_m would result in an increase in the breakdown voltage by a power of two. The independence of the breakdown voltage below a pulse-length of 800 ns can be explained either by a transition from a viscoelastic behaviour of the membrane into the elastic one (i.e., Y_m = constant) or by other processes which are induced within the membrane in response to the field. It has recently been shown [2] that the energy of the ion in the electrical field approximately approaches the Born energy required to pass from the water to the lipid phase. Parsegian [5] has demonstrated that the Born energy is a function of the thickness of the phase with the low dielectric constant when this thickness is less than approx. 4 nm. We cannot exclude, therefore, the possibility that this process plays a role in the breakdown event and becomes increasingly important when the charging times are very short.

In both models the breakdown voltage is also a function of the relative dielectric constant (see Eqns. 1 and 10 in Ref. 2). The dielectric constant is a function of frequency [6, 7] and shows a similar functional relationship to

frequency as observed for the breakdown voltage for lipid bilayers and cells of *V. utricularis* [1]. Thus, it is conceivable that this effect may also become dominant in the nanosecond range of charge-pulse application.

Even though it is impossible to give a completely satisfactory explanation for the pulse-length dependence of the breakdown voltage, this functional relationship is certain to provide a crucial test for the elucidation of the mechanisms of reversible electrical breakdown and probably of mechanical breakdown, since at large pulse lengths the electrical breakdown voltage reaches at 400 mV a value that is close to that of the irreversible mechanical breakdown.

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References

1 Zimmermann, U. and Benz, R. (1979) J. Membrane Biol., in the press
2 Benz, R., Beckers, F. and Zimmermann, U. (1979) J. Membrane Biol. 48, 181—204
3 Benz, R., Stark, G., Janko, K. and Läuger, P. (1973) J. Membrane Biol. 14, 339—364
4 Zimmermann, U. (1978) Annu. Rev. Plant Physiol. 29, 121—148
5 Parsegian, A. (1969) Nature 221, 844—846
6 Schwan, H.P. (1957) in Advances in Biological and Medical Physics (Lawrence, J.H. and Tobias, C.A., eds.), Vol. 5, pp. 147—209, Academic Press, New York
7 Kortüm, G. (1966) in Lehrbuch der Elektrochemie, pp. 138—139, Verlag Chemie, Weinheim
8 Zimmermann, U., Pilwat, G. and Riemann, F. (1974). Biophys. J. 14, 881—899
9 Zimmermann, U., Beckers, F. and Coster, H.G.L. (1977) Biochim. Biophys. Acta 464, 399—416