Biochimica et Biophysica Acta, 382 (1975) 410–418

BBA 76903

DIELECTRIC BREAKDOWN IN THE MEMBRANES OF *VALONIA UTRICULARIS*

THE ROLE OF ENERGY DISSIPATION

HANS G. L. COSTER* and ULRICH ZIMMERMANN

Institute of Physical Chemistry, Nuclear Research Center, Jülich (G.F.R.)

(Received September 27th, 1974)

SUMMARY

The electrical properties of the membranes of *Valonia utricularis* were investigated using intracellular electrodes. Using short (0.5–1.0 ms) current pulses it was found that at a critical membrane potential difference of 0.85 V there was a large and discontinuous decrease in the membrane impedance and the slope resistance beyond this potential was virtually zero.

The electrical breakdown of the membranes did not lead to global damage of the cells and after a resealing time of approx. 5 s could be repeated with identical results.

Experiments with long current pulses and long bursts of pulses repeated at 1 kHz are described which show that the electrical breakdown is not due to thermal damage arising from localized heating in the membrane. Thus a dissipation of some 10^3–10^5 times the energy normally dissipated during the onset of breakdown did not lead to breakdown itself unless the critical membrane potential was exceeded.

The results also show that punch-through and avalanche ionization are not likely to be important in the breakdown mechanism. The results are consistent, however, with there being a critical instability in the electro-mechanical stresses set up in the membrane at large electric field strengths.

INTRODUCTION

It has been shown that electrical breakdown of cell membranes can occur in Coulter Counters with hydrodynamic focussing and electrical discharge chambers [1, 2]. In these experiments dielectric breakdown occurs when the potential difference (PD) between electrodes placed in the cell suspension exceeded a critical value.

Recently, this electrical breakdown phenomenon has also been demonstrated in the giant cells of *Valonia utricularis* using intracellular electrodes [3]. At a critical PD (approx. 0.8 V) there was a discontinuous and very large increase in the membrane conductance and the time constant for this process was of the order of 1 μs. The mechanism for the breakdown is not known, but it did not lead to global, irreversible

* Permanent address: School of Physics, University of New South Wales, Sydney, Australia.

damage to the cell: the breakdown phenomenon could be repeated, on a given cell, many times with identical results.

Short current pulses were used in the experiments, and it is clear that global heating is not likely to be significant in the experiments with intracellular electrodes as also in the Coulter Counter and electrical discharge chamber experiments. In the latter the global heating was determined [4] to lead to temperature increases of between 3 and 5 °C. However, localized heating in the membrane, or more so, in a small region of the membrane, could be perhaps significant.

In the present communication we report results of experiments with cells of *Valonia utricularis*, again using intracellular current and potential electrodes, in which in particular we have investigated the effect of energy dissipation in the membrane to determine the role of any localised thermal damage in the breakdown process. The results presented also allow us to draw some conclusions concerning possible mechanisms involved in the electrical breakdown phenomenon.

METHODS

Cells of the sea water alga *Valonia utricularis*, 3–5 mm long and 2–3 mm in diameter were mounted in an AgCl-coated silver wire grid in a plexiglass chamber. Current was injected into the cells via a platinum/iridium wire electrode (tip approx. 1 μm, shank approx. 5 μm) manipulated longitudinally into the cell through a 5 μm glass micropipette previously inserted into the cell. The Ag/AgCl grid in the (Mediterranean) sea water served as the external current electrode. The membrane PD was monitored with the aid of intra- and extracellular 2 M KCl-filled micropipettes and an electrometer amplifier with capacitance neutralization (a modified "Grass" Inst. model P16). The current was monitored by measuring the potential drop across a 100 Ω resistance in the current circuit. The membrane PD and the current were displayed on a dual beam storage oscilloscope ("Tektronix" 7623). A schematic diagram of the experimental set-up is shown in Fig. 1. All the experiments were done at 20 °C.

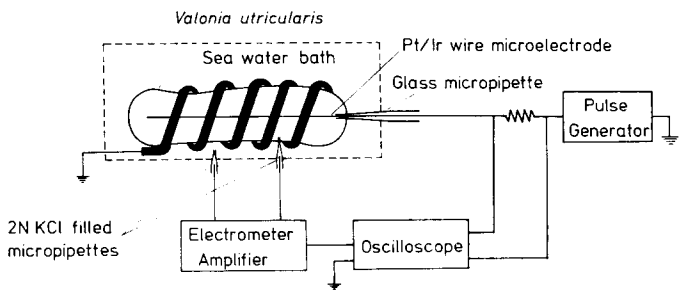

Fig. 1. A schematic diagram of the experimental set-up used in the dielectric breakdown experiments. Current pulses from a pulse generator were passed through the cell membranes. For this the longitudinal platinum/iridium wire electrode, manipulated through the glass micropipette inserted into the cell, and the AgCl-coated silver wire coil, served as the current electrodes. The membrane potential was measured with the 2 M KCl filled, intra and extra cellular, micropipettes and a high input impedance (10^{11} Ω) electrometer amplifier with capacitance neutralization. The current, measured by the potential drop across a series resistance, and the membrane PD were displayed on a storage oscilloscope.

The electrical breakdown PD for a given cell was determined by injecting a series of short (0.2–1.0 ms) current pulses of increasing magnitude into the cell from a pulse generator. The effect of dissipating energy in the membranes was investigated by injecting various long current pulses. For these experiments the pulse height was initially adjusted so that for short pulses the membrane PD remained below the critical breakdown value (hereafter referred to as a subcritical pulse, in contrast to supracritical pulses which lead to breakdown).

Cells of *Valonia utricularis* have a tough, elastic cell wall which does not make it feasible to insert electrodes into the cytoplasm. All the measurements reported here therefore refer to the total cell barrier, plasmalemma-cytoplasm-tonoplast. For simplicity, however, quantities such as PD and resistance will be referred to as membrane PD and membrane resistance.

RESULTS

Short current pulses

The membrane PD measured in a cell for a series of 700 μs current pulses, of increasing magnitude, is shown in the oscilloscope tracing of Fig. 2. Such oscillograms were consistently obtained for all cells examined. It is clear that at a total membrane PD of about 0.85 V (the resting PD for this cell was +6 mV) there was a dramatic and rapid increase in current. This breakdown phenomenon in a given cell could be repeatedly observed, after a delay of approx. 5 s, with quantitatively identical results.

When supracritical pulses were applied too rapidly in succession the cells deteriorated rapidly. Following breakdown, the membrane PD also often decreased

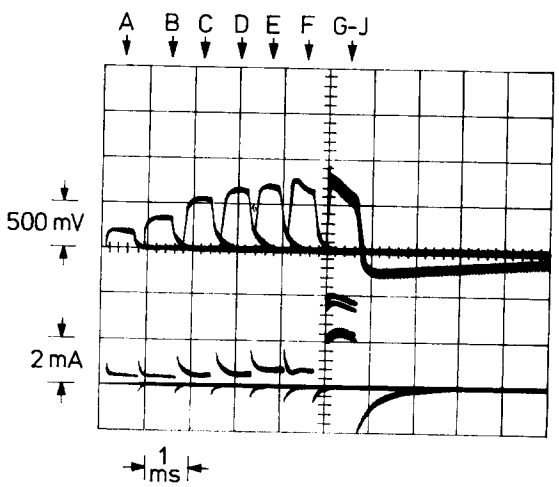

Fig. 2. An oscilloscope tracing of a series of current pulses (lower traces) and the corresponding membrane PD (upper traces), (intracellular potential positive with respect to the external sea water). The time between successive current pulses was about 5 s. They were horizontally displaced, except for the last three pulses, by adjustment of the oscilloscope. Note the large and discontinuous increase in the current at a total PD of approx. 0.85 V (the resting PD for this cell was approx. +6 mV). The last trace for the PD is a superposition of 4 traces, each related to the current pulses shown in the corresponding lower traces.

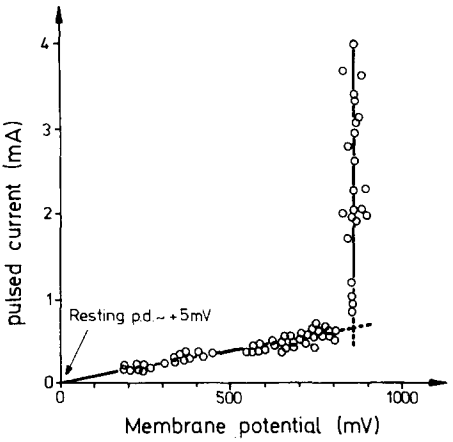

Fig. 3. The pulsed current as a function of membrane potential, vacuole (positive) with respect to outside, for a cell of *V. utricularis*. The data were collected from 13 oscillograms, such as that shown in Fig. 2, obtained in an experiment on one cell. The sharp break in the I-V relation at approx. 0.85 V was observed in all cells examined. At PD values below the breakdown value the membrane resistance was approx. 1300 Ω ($\equiv$ 460 Ω cm^2 for this cell). The potential shown is the total PD, that is the resting potential plus the PD developed across the membrane by the current pulses. The resting PD, however, for this cell was approx. +5 mV and this is therefore negligible compared to the total PD developed during the current pulses. For supracritical pulses the initial membrane PD response is plotted; after breakdown the PD dropped during these supracritical current pulses by approx. 300 mV but the current remained substantially constant (see also text and Fig. 2).

by several millivolts (from a usual value of between +3 and +10 mV). The membrane PD, however, recovered in 10–20 min and if the cell was not subjected to further supracritical pulses during this recovery period the process could be repeated many times.

An example of the cumulative data, collected at approximately 15-min intervals, for the pulsed I-V characteristics of a cell, is shown in Fig. 3. It can be seen that the slope resistance decreased very abruptly at the critical PD from a potential independent value of about 1300 Ω ($\equiv$ 460 Ω cm^2 for this cell) to a very small value.

Once the critical PD is reached the shape of the current and voltage pulses also changed, as is evident from Fig. 2. The initial capacitative current spike present in subcritical pulses is virtually absent in the supracritical pulses. A particularly noteworthy feature of the results shown in Fig. 2 is that during supracritical pulses (pulses G–J in Fig. 2) the membrane PD, following breakdown, decreased, in time, by about 300 mV; well below some of the previously applied subcritical pulses. The current nevertheless remained at the same high value. It would thus appear that following electrical breakdown the membrane resistance remained low during the current pulse and was then no longer dependent on the membrane PD.

Long current pulses

The effect of large, but subcritical pulses, of 10 and 100 ms duration was examined. The results are shown in Fig. 4. On no occasions was a gradual or run-away increase in the current observed. Despite the fact that the current during these long

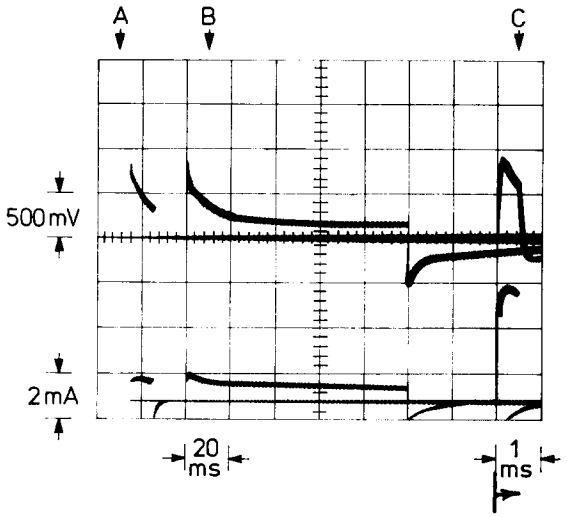

Fig. 4. An oscilloscope tracing of the membrane PD (upper traces) and current (lower traces) for: (A) a subcritical 10 ms pulse, (B) a subcritical 100 ms pulse and (C) a supracritical 500 μs pulse. For the last pulse the timebase of the oscilloscope was expanded (as indicated on the figure).

pulses was substantially constant, the membrane PD gradually decreased*, with a time constant of 1–10 ms, during each pulse, from an initial value of approx. 700 mV to an asymptotic value in the case of 100 ms pulses, of approx. 300 mV. As noted earlier however, this also occurred during supracritical pulses, but then with a decay time constant of approx. 10–100 μs.

Following a large, but subcritical 100 ms current pulse, the cell still displayed the normal breakdown characteristics for short supracritical pulses (Trace C in Fig. 4).

While the membrane PD following breakdown, during a supracritical current pulse, might decrease to values similar to those during the previous 100 ms subcritical pulses (Trace B in Fig. 4), it can be seen that the current in the supracritical pulses remained much larger than those in the long subcritical pulses. Similarly, 10 s bursts of subcritical 0.7 ms pulses which took the membrane to a PD of 800 mV at a repetition rate of 1 kHz also did not lead to electrical breakdown (Fig. 5). Again, following this long burst of subcritical pulses, dielectric breakdown occurred when a single supracritical pulse of current was applied (last pulse shown in Fig. 5).

DISCUSSION

The mechanism responsible for the electrical breakdown here reported appears to be critically dependent on the membrane PD; it is clear from the results shown in both Figs 2 and 3 that the cell cannot be polarized beyond 0.85 V, even for large

* Similar effects with current pulses which lead to much smaller excursions of the membrane PD, have long been noted in other cell membranes so examined. These effects, which are related to a concentration polarization due to transport number differences in the membrane and external solutions, have been discussed in detail in the literature (e.g. see refs 5 and 6).

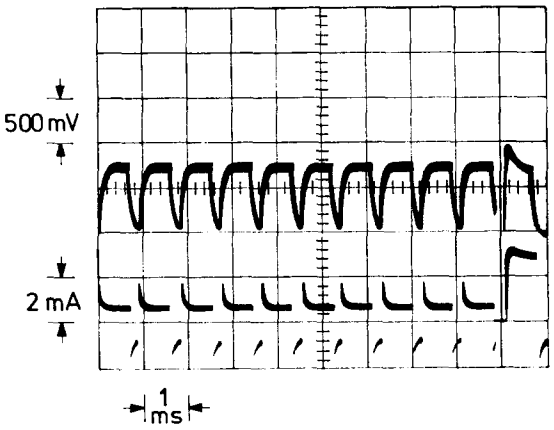

Fig. 5. An oscilloscope tracing of the membrane PD (upper trace) and current (lower trace) for a 10 s burst of 700 μs current pulses injected into the cell. Note that the membrane PD was approx. 800 mV during each pulse, but no electrical breakdown occurred. Following this burst the cell yielded the usual result for a single supracritical pulse (last pulse shown).

current pulses. The electrical breakdown phenomenon observed with hydrodynamic focussing Coulter Counters and electrical discharge chambers was similarly critically dependent on the PD between the electrodes placed in the cell suspensions. Solution [2, 7] of the Laplace equation for the distribution of the electrical potential in such cell suspensions showed that breakdown occurred when the PD generated across the membrane was ≈ 1.4 V for bovine erythrocytes and 1.1–1.3 V for cells of *Escherichia coli B*. Considering the approximations that had to be made to obtain numerical solutions of the Laplace equation, these values are not very different from the value presently determined by the more direct measurements in *V. utricularis*.

Four possible mechanisms which could lead to such a property will now be considered. However, only the last of these seems at all plausibly consistent with the present experimental findings.

1. Punch-through

It is evident from the results such as those shown in Fig. 2 that the increase in current associated with electrical breakdown occurs in a very short time (1 μs or less). This is in agreement with the Coulter Counter and electrical discharge experiments where the total pulse lengths were only 20 μs and 5–10 μs, respectively [2, 7]. On the other hand it is clear from diffusion polarization processes, such as those revealed by studies of the very low frequency (< 100 Hz) capacitance dispersion [8], that ionic diffusion effects in the membranes of algal cells have time constants of the order of 0.01–1.0 s. Ionic punch-through is associated with changes in the ion concentration profiles in the membrane and ionic currents [9–11]. It is thus clear that the electrical breakdown here described is qualitatively different from the punch-through effect. This is further substantiated by the fact that in the Coulter Counter and electrical discharge chamber experiments dielectric breakdown was associated with haemoglobin release [2, 4, 12]; a result not consistent with it being due to punch-through.

2. Avalanche production of charge carriers

The voltage current characteristic shown in Fig. 3 are very reminiscent of those of a semiconductor Zener diode. Because of the non crystalline nature of the cell membrane it is not likely that a similar mechanism is responsible for the electrical breakdown. The current after breakdown is sustained despite the fact that the PD dropped well below the critical value (see traces G–J in Fig. 2). Avalanche production of charge carriers could then not continue unless the quenching processes are particularly weak. The latter, however, is not the case; in the plasma membrane ions have mobilities which are about 10^{-3} or less of that in an aqueous solution.

3. Localised thermal membrane damage

The breakdown phenomenon is critically dependent on the membrane PD. It would seem unlikely that such a sharp dependence on membrane potential would result if the breakdown is due to localised thermal damage in the membrane, unless the, pulsed, I-V characteristic for these membranes was extremely non-linear. In fact up to the critical potential the I-V characteristic appeared to be very linear (see Fig. 2). Further, during long, but subcritical, pulses no run-away decrease in the membrane resistance was observed, irrespective of the pulse length used.

The power dissipated during such current pulses can be calculated from oscilloscope tracings such as those shown in Fig. 4. Examples of the calculated power dissipated is shown in Figs 6A and 6B. Note the different time scales used in these

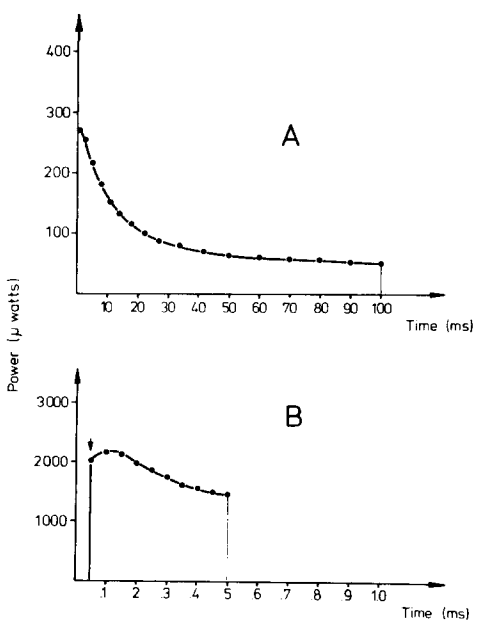

Fig. 6. The power dissipated during current pulses injected into a cell (A) a 100 ms subcritical pulse. The area under this curve represents an energy of 8.6 μJ. (B) an approx. 500 μs supracritical pulse. Here the total area under the curve amounts to 0.92 μJ. Only the area covered by the thickness of the vertical line, indicated by the arrow, represents the upper limit for the energy dissipated during the onset of breakdown and amounts to only $9.5 \cdot 10^{-3}$ μJ.

figures. The initial instantaneous power in the short supracritical pulse is larger than that dissipated in the longer subcritical current pulses, but the total energy dissipated in these long pulses far exceeds that during the supracritical pulses. Thus during the 100 ms current pulse the total energy dissipated in the membrane amounted to 8.6 μJ. The total energy injected during the 10 s burst of subcritical pulses shown in Fig. 5 amounts to 2710 μJ. In contrast during the whole of the supracritical pulse, only 0.92 μJoules of electrical energy was injected into the cell. This figure, however, is a gross overestimate of the energy dissipated during the onset of electrical breakdown.

As has been pointed out above the breakdown process occurs over a time of 1–5 μs. Hence from the power curve shown in Fig. 6B the upper limit for the amount of energy dissipated during the breakdown process (area covered by vertical line indicated in Fig. 6) is then $2\text{–}10 \cdot 10^{-3}$ μJ, that is, some 3 orders of magnitude less than the energy dissipated during the 100 ms subcritical current pulse shown in Fig. 4 and 5 orders of magnitude smaller than the 10 s burst (at 1 kHz) of 700 μs subcritical pulses (Fig. 5). Neither of the latter two lead to breakdown. However, it seems not unlikely that once breakdown has occurred by whatever mechanism, thermal effects may play an important role in maintaining a low resistance. This is so because electrical breakdown probably occurs in a small region(s) of the membrane and most of the current would then flow through this region(s).

4. Electro-mechanical instabilities

From the previous discussions we must conclude that the present results indicate a breakdown, at least locally, of the dielectric properties of the membrane. This may be simply a field induced mechanical instability of the membrane as recently discussed by Crowley [13]. Thus the presence of an electric field creates a stress which leads to a mechanical compression of the membrane material. On the assumption of a linear potential gradient, for a given membrane PD, the electrical stress induced increases as the inverse square of the membrane thickness. The elastic restoring stress, however, increases only logarithmically with the strain. At a critical PD, which depends on the elastic modulus and the dielectric constant, a catastrophic reduction of the membrane thickness will occur. This is consistent with the breakdown here described. With reasonable values of these parameters the critical potential is of the order of that reported here [4]. It is also in agreement with the observation that once breakdown has occurred this state is no longer critically dependent on the potential. This mechanism is further not inconsistent with the high speed of the breakdown process observed.

CONCLUSIONS

From the results obtained we can conclude:

1. Electrical breakdown in cells of *Valonia utricularis* occurs at a critical membrane PD of about 0.85 V.
2. This breakdown phenomenon is very similar to that observed in erythrocytes, bacteria and algal cells in hydrodynamic focussing Coulter Counters and electrical discharge chambers.
3. The electrical breakdown does not lead to global damage of the cell and after approx. 5 s the membrane region where breakdown has occurred is restored. The process, in a given cell, can be repeated many times.

4. The electrical breakdown is not due to thermal damage in the membranes.
5. The breakdown of the dielectric properties of the membrane is consistent with considerations of electro-mechanical instabilities of the membranes.

ACKNOWLEDGEMENTS

U. Zimmermann wishes to thank the Deutsche Forschungsgemeinschaft for a research grant (Sonderforschungsbereich 160). H. G. L. Coster wishes to thank the KFA Jülich and the University of New South Wales (Sydney, Australia) for support to conduct this research. The authors are grateful to W. Petrick and E. Klein for very skillful technical assistance.

REFERENCES

1 Zimmermann, U., Schultz, J. and Pilwat, G. (1973) Biophys. J. 13, 1005–1013
2 Zimmermann, U., Pilwat, G. and Riemann, F. (1974) Biophys. J. 14, 881–889
3 Coster, H. G. L. and Zimmermann, U., Z. Naturforsch., in the press
4 Zimmermann, U., Pilwat, G. and Riemann, F. (1975) Biochim. Biophys. Acta, 375, 209–219
5 Barry, P. H. and Hope, A. B. (1969) Biophys. J. 9, 700–728
6 Läuger, P. and Neumcke, B. (1973) in Membranes: A series of Advances (Eisenmann, G., ed.), Marcel Dekker, Inc., New York
7 Zimmermann, U., Pilwat, G. and Riemann, F. (1974) in Membrane Transport of Plants (Zimmermann, U. and Dainty, J., eds), Springer, Heidelberg
8 Coster, H. G. L. and Smith, J. R. (1974) in Membrane Transport of Plants (Zimmermann, U. and Dainty, J., eds), Springer, Heidelberg
9 Coster, H. G. L. (1965) Biophys. J. 5, 669–686
10 Coster, H. G. L. (1969) Aust. J. Biol. Sci. 22, 365–374
11 Coster, H. G. L. and Hope, A. B. (1968) Aust. J. Biol. Sci. 21, 243–254
12 Zimmermann, U., Pilwat, G. and Riemann, F. (1974) Z. Naturforsch. 29, 304–305
13 Crowley, J. M. (1973) Biophys. J. 13, 711–724