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Transport Properties of Mobile Charges in Algal Membranes: Influence of pH and Turgor Pressure

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Summary. Charge-pulse experiments were performed on giant algal cells of *Valonia utricularis*. If the tonoplast and plasmalemma in series are charged to voltages of the order of 10 mV, the decay of the initial voltage with time can be described by the sum of two or three exponential relaxations. It is not possible to explain the exponential decay of the voltage by two *RC*-circuits in series (e.g. tonoplast and plasmalemma), because this would lead to unreasonable values for the specific capacities of the two membranes. The exponential relaxations might be attributable to the transport of mobile negative charges present in both membranes, possibly as a part of a transport system. From an analysis of the experimental results in terms of the proposed model, the translocation rate constant k and the total surface density N_t of the mobile charges in one membrane could be evaluated. On average k is of the order of $600\,sec^{-1}$ and N_t is about $5\times10^{-12}\,mol\,cm^{-2}$ (average turgor pressure 1.6 bar). The transport properties of the mobile charges within the tonoplast and plasmalemma were studied as a function of different parameters such as external pH, glutardialdehyde, electrical breakdown and turgor pressure. When the pH is lowered from 8.2 to 4 or 5 the mobile charges disappear completely, presumably as the result of protonation of the anionic groups. This pH effect was found to be completely reversible. Electrical breakdown causes a reversible disappearance of the relaxation with the longer half-time due to the decrease in membrane resistance. The value of the electrical breakdown voltage determined by injection of charge pulses of 300-μsec duration into the cell is pH-independent and therefore is consistent with the mobile charge model and with results previously reported (U. Zimmermann & R. Benz, *J. Membrane Biol.* **53**:33–43, 1980). Addition of glutardialdehyde leads also to a disappearance of the mobile charges probably due to cross-linkage. Increase of the turgor pressure from 0.05 bar to 2 bar results in an increase in k by a factor of 2 and in N_t by about 30%. The increase in k is in reasonable agreement with that expected on the basis of the assumed compressibility of the membranes. The elastic compressive modulus perpendicular to the membrane plane calculated from the pressure dependence of the translocation rate constant k is in very good agreement with that derived from electrical breakdown experiments (14 and 13 bar, respectively). The presence of charges within the membranes as well as the compressibility of the membranes are discussed in terms of a possible turgor-pressure-sensing mechanism.

Key words voltage relaxations · mobile charges · turgor-pressure-sensing mechanism · *Valonia*

Introduction

There is a body of evidence that membrane transport and certain metabolic processes in the cytoplasm are either under control of turgor pressure or at least turgor-pressure-dependent [9, 15–18, 24–27, 37–39]. The control of cell turgor pressure in response to osmotic stress or during growth is therefore a prerequisite for a plant cell to maintain its functional state. The efficiency of plant cells in maintaining a constant turgor pressure over a wide range of external osmolarities is particularly apparent in cells living in environments of fluctuating salinity (e.g. estuaries).

The mechanism by which a turgor-pressure signal in response to osmotic stress is sensed by the cell membrane is unknown. Zimmermann and colleagues [12, 13, 25, 28, 29, 34] have proposed an electromechanical model based on the assumption that a change in the membrane thickness brought about by turgor-pressure changes is the primary step in the transformation of a turgor-pressure signal into alterations of the biophysical and biochemical properties of the membrane. The key prediction of this quantitative model is that the intrinsic electric field within the membrane is initially altered by changes in the membrane thickness. Thus, changes in the electric field or in the concentrations of ions in the surrounding medium which contribute to the membrane potential should also lead to a control of cell turgor. This and several other predictions of the model have been verified experimentally in the meantime [20, 27, 33], supporting the idea that the key assumption of the model, which was calculated on the basis of macroscopic laws, is valid.

Changes in the membrane thickness can be brought about by direct compression of the mem-

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brane or indirectly by stretching of the plasmalemma coupled to the cell wall. Changes in the membrane thickness arising from stretching can be estimated from the measurements of the elastic coefficient of the cell wall, whereas an estimate of the elastic compressive modulus perpendicular to the membrane plane can be obtained from electrical-breakdown experiments [32]. For the giant algal cells of *Valonia utricularis* it was calculated that the membrane thickness can be reduced by about 0.2 to 0.4 nm when the turgor pressure is changed by 1 bar [25].

In this communication we present further evidence supporting the postulated turgor-pressure-sensing mechanisms by using a quite different experimental and theoretical approach. The membranes were charged very rapidly (within nsec) to a low voltage at different turgor pressures and the dependence of the voltage relaxation within the membrane on turgor pressure was subsequently measured [4–8][1]. It has emerged from these experiments that the voltage relaxation has at least two components: a long relaxation, which is usually six to ten times longer than the normal RC-relaxation, and a second fast (and very often a third intermediate) relaxation arising from the movement of mobile charges within the membrane due to carrier systems. The surface density of these mobile charges is very high and the translocation rate of the charges within the membrane, as well as the density, are pressure-dependent. The increase in the translocation rate with increasing turgor pressure, particularly in the pressure range in which the potassium fluxes of *V. utricularis* are markedly pressure-dependent [22], can be explained by a change in the membrane thickness. This is supported by analogous experiments on lipid bilayer membranes of variable thickness in which lipophilic ions had been introduced [4]. The calculated value for the change in membrane thickness with turgor pressure is in very good agreement with the value obtained from electrical breakdown experiments and analysis of these data on the basis of the electromechanical model [25]. Thus, it is suggested that turgor pressure is sensed both by a change in membrane thickness and by the presence of mobile charges within the membrane.

Materials and Methods

Cells of *V. utricularis*, originally collected in Naples, Italy, were grown in natural seawater at a salinity of 1150 mosmol kg^{-1} as described earlier [39]. Elliptical cells with a volume between 18 and 100 µl were fixed in a small perspex chamber continuously perfused with seawater. The temperature of the circulating seawater was monitored by a thermistor mounted approximately 2 mm away from the cell. All experiments were performed at 17 °C in natural seawater at a pH of about 8.2. The solutions of pH 4 or pH 5 were buffered with 10 mM citrate. The turgor pressure of the cells used for this study was measured with the pressure probe [25–27, 37, 38]. The normal turgor pressure of the cells was 1 to 2 bar. It was varied between 0.05 and 3 bar by adding either NaCl (analytical grade) or distilled water to the circulating external solution. The resting membrane potential was about 2 mV (inside positive).

In the charge-pulse experiments the membrane capacitance of the algal cells was charged to voltages between 6 and 15 mV by injecting short current pulses (50 to 200 nsec duration) generated by a fast-pulse generator (Hewlett Packard 214 B, maximum output voltage 100 V at 50 Ω). The generator was connected to the internal current electrode (platinized platinum wire) through a diode with a reverse biassed resistance larger than 10^{10} Ω. The actual voltage across the membrane was measured using a second internal electrode (silver/silver chloride wire) connected to the input of a Tektronix 7633 storage oscilloscope (7A22 amplifier, 1 MΩ input resistance, maximum band-width 1 MHz) driving a Nicolet Instruments Explorer III digital storage oscilloscope (Model 206 plug-in, maximum sampling rate 2 MHz). A voltage follower (using a Burr Brown 3551 operational amplifier, input resistance 10^{12} Ω) was used to test if the input resistance of the oscilloscope had any influence on the long time constant τ_2. In most experiments described here the decrease of τ_2 was less than 3%, whereas it was less than 10% in all experiments. A silver/silver chloride electrode of extremely large surface area was mounted close to the cell and was used as an external voltage and current electrode. The resistance of the current electrode was on the order of 100 Ω, whereas the voltage electrode had a resistance around 1 kΩ. Both electrodes had an effective capacitance of less than 100 pF. The whole arrangement was similar to one published earlier [8].

The injected charge in the charge-pulse experiments was measured using the voltage drop across a 10 Ω resistor in series with the external electrode. The charge was determined by planimetry of the area below the voltage trace.

The whole apparatus was carefully checked with test circuits as described earlier [1, 30]. One of these experiments is presented in Fig. 1. A capacitor of 0.47 µF in parallel with a resistor of 1 kΩ was connected in series with the internal and external current electrode (R_e) (*see inset* in Fig. 1) and the decay voltage in the test circuit was measured after a charge pulse. The time resolution of the whole apparatus was of the order of 5 µsec. The data obtained from the digital oscilloscope was analyzed directly with a HP-9825 A or a MINC-11 computer, whereas photographs of the oscillographic records were digitized with a Summagraphics Digitizer (HV-2-20). In this latter case the analysis of the data was performed with a HP-9820 A computer.

Electrical breakdown experiments were performed using this experimental arrangement as described earlier [30].

The actual resistance of the whole cell was measured with this set-up by injecting a constant current of 1 to 30 µA into the cell for about 20 msec. The voltage drop across both membranes and the constant current were recorded. The specific resistance of the cells was calculated according to Ohm's law and was normally of the order of 500 to 4000 Ω cm^2.

In a parallel series of experiments the dependence of the current-voltage properties of the membranes on turgor pressure was measured in the conventional way, i.e. glass microelectrodes filled with 3 M KCl were used for measuring the membrane potential and for applying the current from a constant current source (Keithley 225). The duration of the injected current pulses was 5–

[1] Benz, R., Zimmermann, U. Evidence of mobile charges in cell membranes of *V. utricularis* (*in preparation*)

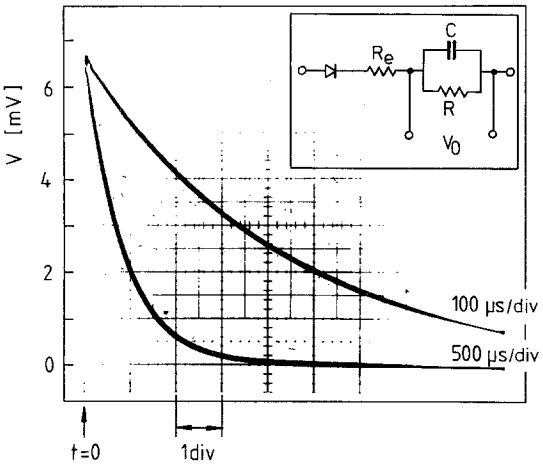

Fig. 1. Oscilloscope record of a control experiment on the electrical analog circuit shown in the inset. The charge pulse of 200-nsec duration and 2.9×10^{-9} A sec injected charge Q resulted in an initial voltage $V_0 = 6.5$ mV across the parallel combination of resistor (1 kΩ) and capacitor (0.47 µF) simulating the membrane. The voltage relaxation is purely exponential with a time constant τ of 430 µsec. To provide better data it was recorded twice at different time resolutions. C and R of the analogous circuit were calculated to be 0.45 µF and 964 Ω according to $Q = C \cdot V_0$ and $\tau = R \cdot C$

10 sec. The membrane resistance was calculated from the linear part of the current-voltage relationship. For further details *see* [36].

The vacuolar potassium influx was measured by the uptake of $^{42}K^+$ added to the seawater. The procedure and the calculation of the influx from the uptake of labeled potassium into the vacuole was performed as described elsewhere [22].

Some voltage-relaxation measurements were performed on cells fixed with glutardialdehyde (Merck, FRG). Four ml of 25% glutardialdehyde solution were added to 100 ml seawater at pH 8.2 whereby a final concentration of 1% (100 mM/liter) was established.

Theoretical Considerations

Only general features of the considered transport system will be described here. The model will be given in full detail elsewhere[2]. It is based on the assumption of a mobile carrier system similar to that used for the kinetic analysis of proton transport mediated by uncouplers of the linear type [3, 6]. It assumes that a chemical reaction (complex formation) takes place between a membrane-bound anionic group A^- and a positively charged substrate molecule S^+ (for example protons or potassium ions) at the membrane/solution interface (Fig. 2). This chemical reaction is described by overall rate constants, k_R^{AS} (association) and k_D^{AS} (dissociation). The translocation of the neutral complex AS

[2] *See* footnote 1, p. 184.

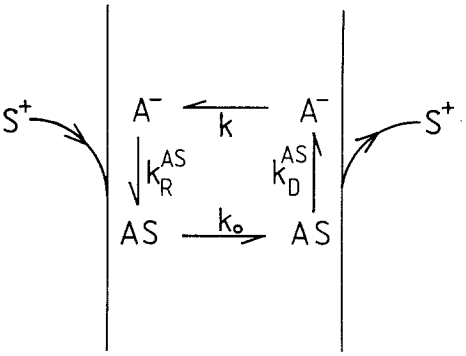

Fig. 2. Model for the transport of the substrate S^+ by the anionic group A^-

through the membrane is treated as a first-order reaction with the rate constant k_o. The translocation of the anionic group A^- through the membrane is described in a similar way by the rate constant k. This rate constant is assumed to be voltage-dependent, denoted by k' and k''.

Furthermore it is assumed that the two membranes in series, tonoplast and plasmalemma, separate identical concentrations of the transported cation S^+ (concentration C_S). This is not a serious restriction of the theoretical treatment if the only driving force for the substrate transport is the voltage difference across both membranes. This voltage (i.e. resting potential) is normally of the order of 2–5 mV (inside positive) and would lead to an almost identical concentration of the substrate on both sides of the membrane barrier. The system is in equilibrium at times $t<0$. At time $t=0$ the membrane capacitance C_m is charged within nanoseconds to a voltage V_m^0.

Denoting the surface concentrations of A^- and AS at the outside interface by N'_A and N'_{AS} and the surface concentrations at the inside interface by N''_A and N''_{AS}, the derivatives of these quantities are given by:

$$\frac{dN'_A}{dt} = -k_R^{AS} \cdot C_S \cdot N'_A + k_D^{AS} \cdot N'_{AS} - k' \cdot N'_A + k'' \cdot N''_A, \quad (1)$$

$$\frac{dN''_A}{dt} = -k_R^{AS} \cdot C_S \cdot N''_A + k_D^{AS} \cdot N''_{AS} + k' \cdot N'_A - k'' \cdot N''_A, \quad (2)$$

$$\frac{dN'_{AS}}{dt} = k_R^{AS} \cdot C_S \cdot N'_A - k_D^{AS} \cdot N'_{AS} - k_0 \cdot N'_{AS} + k_0 \cdot N''_{AS}, \quad (3)$$

$$\frac{dN''_{AS}}{dt} = k_R^{AS} \cdot C_S \cdot N''_A - k_D^{AS} \cdot N''_{AS} + k_0 \cdot N'_{AS} - k_0 \cdot N''_{AS}. \quad (4)$$

The total carrier concentration in the membrane (either complexed or uncomplexed) should be time-independent (at least within the time scale of a charge-pulse experiment), i.e.

$$N'_A + N''_A + N'_{AS} + N''_{AS} = N_t. \tag{5}$$

The rate of decay of the voltage V_m following the charge pulse is given by the current density J through the membrane. J is associated with the flow of charges, the carrier system and the unspecific ohmic conductance R_m of the membrane:

$$J = -F(k' \cdot N'_A - k'' \cdot N''_A) - \frac{V_m}{R_m}. \tag{6}$$

With $J = \frac{dQ_m}{dt} = \frac{dV_m}{dt} \cdot C_m$, Eq. (6) becomes:

$$\frac{dV_m}{dt} = -\frac{F}{C_m} \cdot (k' \cdot N'_A - k'' \cdot N''_A) - \frac{V_m}{R_m C_m}. \tag{7}$$

From the experimental relaxation curves it is obvious (*see* Results) that either the substrate concentration C_S in the aqueous phase is extremely low ($N_A \gg N_{AS}$) or the complexation reaction is very slow ($k \gg k_R^{AS}, k_D^{AS}$). In both cases only two relaxations would be observed [5]. The total surface concentration $N_t = N'_A + N''_A$ calculated under these conditions is of the order of 5×10^{-12} mol cm^{-2} for a single membrane (*see below*). This means that one membrane surface contains about 1 charge per 33 nm^2. The concentration of charges is then only 60 times lower than the density of lipid molecules in a pure lipid bilayer (1 lipid molecule per 0.6 nm^2). Because of this high surface concentration of charges, it seems to us more likely that the substrate concentration C_S is low (N'_A, $N''_A \gg N'_{AS}$, N''_{AS}). Then the transport kinetics of the anionic groups A^- within the membrane can be expressed in a similar way to the transport of lipophilic ions [2, 5] and gating particles [2] in membranes (two-state model). In this case the derivatives of the surface concentrations N'_A and N''_A with time reduce to [*compare* Eqs. (1) and (2)]:

$$\frac{dN'_A}{dt} = -k' \cdot N'_A + k'' \cdot N''_A, \tag{8}$$

$$\frac{dN''_A}{dt} = -k'' \cdot N''_A + k' \cdot N'_A, \tag{9}$$

$$N_t = N'_A + N''_A \tag{10}$$

where k' and k'' are voltage-dependent according to

$$k' = k \cdot e^{u/2}, \tag{11}$$

$$k'' = k \cdot e^{-u/2} \tag{12}$$

and where u is the reduced voltage

$$u = \frac{V_m \cdot F}{R \cdot T}. \tag{13}$$

In the limit of low voltages $u < 1$ ($V_m < 25$ mV) Eqs. (7)–(9) represent a system of two linear differential equations with the following solution for $V_m(t)$ [2][3]:

$$V_m(t) = V_m^0(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}) \tag{14}$$

where

$$a_1 + a_2 = 1. \tag{15}$$

a_1 and a_2 are the relative amplitudes of the two exponential decays.

Defining the quantities Z_1, Z_2 and Z_3 as [2][4]:

$$Z_1 = \frac{1}{\tau_1} + \frac{1}{\tau_2}, \tag{16}$$

$$Z_2 = \frac{1}{\tau_1} \cdot \frac{1}{\tau_2}, \tag{17}$$

$$Z_3 = \frac{a_1}{\tau_1} + \frac{a_2}{\tau_2}. \tag{18}$$

k, R_m and N_t can be calculated according to:

$$k = \tfrac{1}{2}(Z_1 - Z_3), \tag{19}$$

$$R_m = \frac{1}{C_m} \cdot \frac{2k}{Z_2}, \tag{20}$$

$$N_t = \frac{1}{2k \cdot b}\left(Z_1 - 2k - \frac{Z_2}{2k}\right) \tag{21}$$

with

$$b = \frac{F^2}{4RTC_m}. \tag{22}$$

In this theoretical treatment it has been assumed that the two membranes in series, tonoplast and plasmalemma, behave in principle like a single membrane. This is justified in our opinion by the fact that the experimental data can be fitted with high precision to the two major exponential relaxations (*see below*). If the two membranes can be treated as one, the assumption of 0.5 μF cm^{-2} for the specific membrane capacity yields the correct value for R_m (sum of the specific resistances of the two single

[3] *See* footnote 1, p. 184.
[4] *See* footnote 1, p. 184.

membranes) but only half of the total concentration N_t of charged particles present in a single membrane. On the other hand, the use of $1\,\mu F\,cm^{-2}$ for the specific capacitance gives the correct value for N_t and the specific resistance of one membrane. R_m is then obtained by doubling the resistance value.

Results

Voltage Relaxation

A typical voltage-relaxation experiment on a cell of *Valonia utricularis* is shown in Fig. 3. The membrane capacitance was charged by a short current pulse of 200-nsec duration to about 7 mV (injected charge 2.8×10^{-9} Asec). As indicated in Fig. 3, the potential difference across the membrane decays in two exponential relaxations with distinct time constants. A semilogarithmic plot of the data is given in Fig. 4. The fast relaxation with a relative amplitude a_1 of 0.88 has a time constant of 110 μsec, whereas the second, slower one (relative amplitude $a_2 = 0.12$) has a time constant of 4.5 msec.

In principle, one can argue that the two relaxations are attributable to the two membranes arranged in series. However, this explanation can be rejected for the following reason. It can be shown that the following equation holds if two membranes arranged in series are charged to a certain voltage V_m^0 by a short charge pulse (200 nsec) duration and of an injected charge Q:

$$Q = C_{m_1} \cdot V_{m_1}^0 = C_{m_2} \cdot V_{m_2}^0 \tag{23}$$

with

$$V_{m_1}^0 + V_{m_2}^0 = V_m^0. \tag{24}$$

C_{m_1} and C_{m_2} are the capacitances of the tonoplast and plasmalemma membrane, respectively, and $V_{m_1}^0$ and $V_{m_2}^0$ are the corresponding initial voltages.

Taking $V_{m_1}^0$ and $V_{m_2}^0$ from Figs. 3 and 4 and the membrane area determined by geometric means ($A = 0.79\,cm^2$) leads to values for C_{m_1} and C_{m_2} of $0.55\,\mu F\,cm^{-2}$ and $4.1\,\mu F\,cm^{-2}$, respectively. Both values are in contradiction to the value of $1\,\mu F\,cm^{-2}$ reported in the literature for the specific capacitance of membranes (e.g. [21]).

On the other hand, the value of $0.48\,\mu F\,cm^{-2}$ for the total specific capacitance of both membranes is in good agreement with the value expected for two membranes arranged in series both exhibiting a specific capacitance of $1\,\mu F\,cm^{-2}$. If we analyze the two voltage decays in terms of two RC relaxations with the time constants

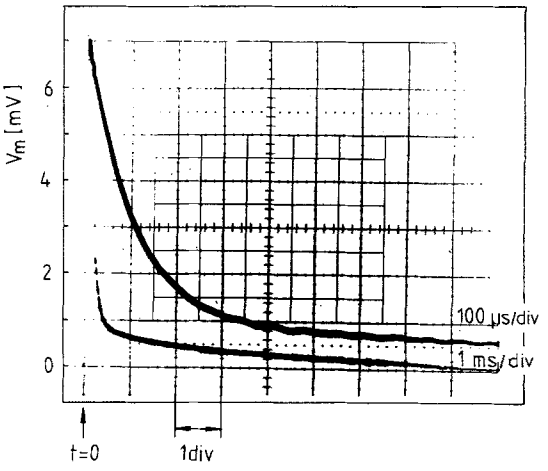

Fig. 3. Voltage-relaxation curve of a typical cell of *Valonia utricularis*, recorded at cell volume 49 μl, turgor pressure 1 bar, natural seawater, pH 8.2. A charge pulse of 200 nsec was injected into the cell (2.8×10^{-9} A sec); $T = 17\,°C$. The relaxation was recorded at two different time resolutions to provide better data

$$\tau_1 = R_1 \cdot C_1 \quad \text{and} \quad \tau_2 = R_2 \cdot C_2 \tag{25}$$

the specific resistances are calculated to be $R_1 = 200\,\Omega\,cm^2$ and $R_2 = 1100\,\Omega\,cm^2$. These values agree with the total specific resistance obtained from measurements of the current voltage relationship ($R_1 + R_2 = R_m = 1400\,\Omega\,cm^2$).

In the light of the very unusual values for the individual specific capacitances of the two membranes and on the basis of experiments on artificial lipid bilayer membranes doped with lipophilic ions [4, 5], we conclude that the two relaxation processes arise from the presence of mobile charges within both membranes which are probably part of a carrier system for positively charged substrates. The analysis of the data in terms of mobile charges is given in the previous section. The theory yields the membrane resistance, as well as the rate constant k of translocation and the total concentraion N_t per unit membrane area of the charged particles in one single membrane. Thus, the value of N_t has to be doubled when using the total specific capacity of $C_m = 0.5\,\mu F\,cm^{-2}$. On the other hand, the theory yields the sum of the resistances $R_m = R_1 + R_2$ when using this value for the specific capacity. For the data presented in Figs. 3 and 4 the following values have been calculated: $k = 630\,sec^{-1}$, $N_t = 4.9 \times 10^{-12}$ mol cm^{-2} and $R_m = 1250\,\Omega\,cm^2$ (turgor pressure $P = 1$ bar).

The value for R_m obtained from the analysis of the voltage-relaxation curves on the basis of the charge model agrees well with that deduced from

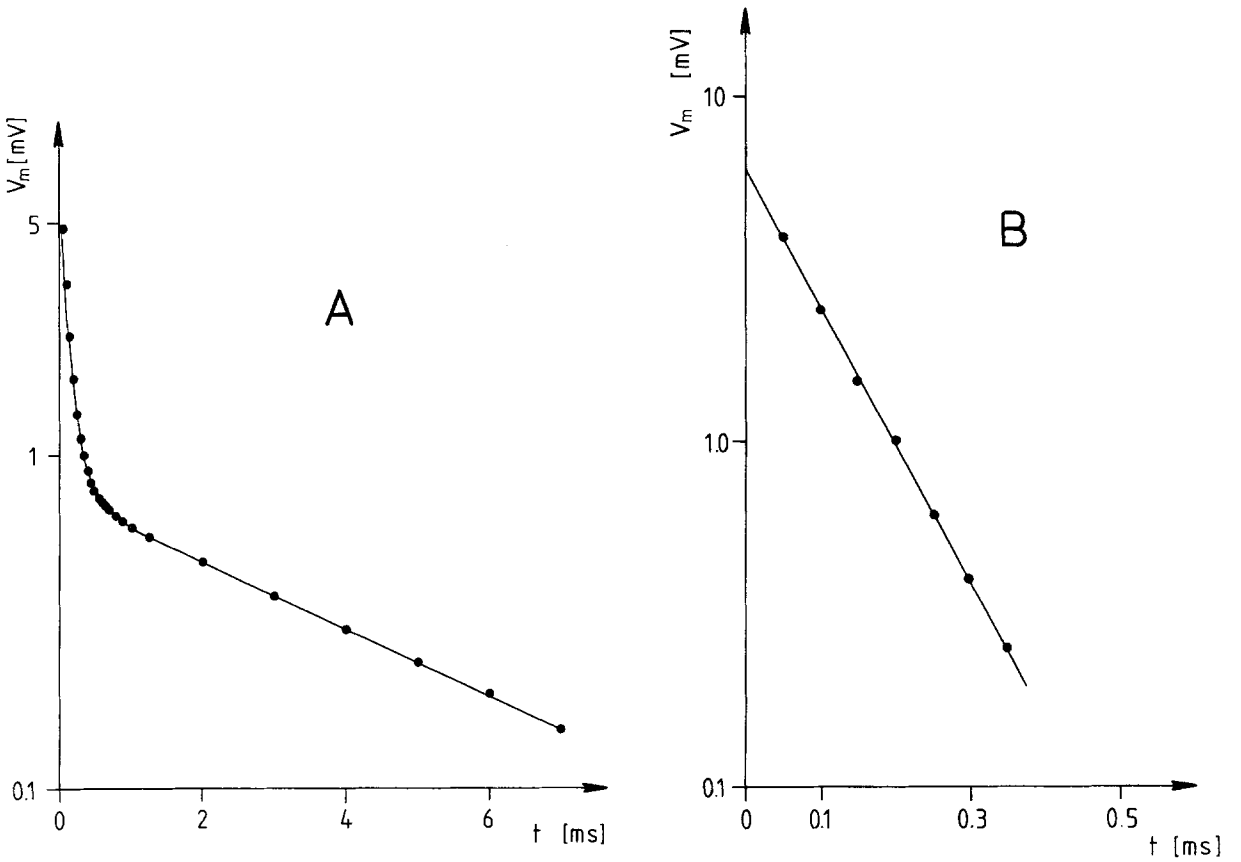

Fig. 4. Semilogarithmic plot of the voltage *vs.* time of the experiment given in Fig. 3. The initial voltages $V_{m_1}^0$ and $V_{m_2}^0$ as well as the time constants τ_1 and τ_2 were calculated using the least-squares method. (*A*) Semilogarithmic plot of the total relaxation process. (*B*) Semilogarithmic plot of the difference between the data at fast times and the fitted record relaxation process (V_{m_1}, τ_1) $V_{m_1}^0 = 6.5$ mV; $\tau_1 = 110$ µsec; $V_{m_2}^0 = 0.86$ mV; $\tau_2 = 4.5$ msec. From the relaxation data the following values for kinetics of charge movements were calculated: $k = 630\,\text{sec}^{-1}$; $N_t = 4.9 \times 10^{-12}\,\text{mol cm}^{-2}$; $R_m = 1250\,\Omega\,\text{cm}^2$ ($C_m = 0.5\,\mu\text{F cm}^{-2}$)

injecting a constant current into the cell and measuring the voltage drop across the membrane ($R_m = 1400\,\Omega\,\text{cm}^2$) (*see also* ref. [39]).

The values for these three parameters obtained from 21 independent experiments at initial turgor pressure are listed in Table 1. The variation in the absolute value of these three parameters may arise both from the differences in the physiological state of the *Valonia* cells (as has been found for other membrane properties [28]), but also from the pressure dependence of these parameters (*see below*).

In the analysis of the data obtained with the digital oscilloscope Explorer III (4096 points, 12 bit resolution), a third intermediate relaxation of about 5% of the total amplitude and a time constant of 300 to 1000 µsec was detected in about 50% of the experiments. An example of such an analysis is given in Fig. 5. The third relaxation is not included in the theoretical analysis of the transport system. It is not clear whether this relaxation is involved in transport of the ionic groups A^- (or its neutral complex AS) or is due to other transport systems present in one or both membranes. In addition, it is assumed that the charged groups have the same translocation rate constant in both membranes and that both membranes have the same resistance. If this is not the case, i.e. if k or R_m differ for both membranes by 20 to 30%, this could be the cause of the intermediate relaxation.

In view of these considerations and the fairly small influence of the intermediate relaxation on the results, only the fast and the slow relaxations have been used in the following for the calculation of the data. This is also justified by the fact that the kinetics of the charge movement in a cell with two relaxations and a cell with three relaxations (the intermediate relaxation neglected) do not show any specific difference.

Influence of pH and Glutardialdehyde

Measurements in which the pH of the natural seawater bathing the cell was lowered from pH 8.2 to

Table 1. Analysis of voltage relaxations in terms of the mobile charge model

Alga	V (µl)	A (cm^2)	P (bar)	a_1	τ_1 (msec)	τ_2 (msec)	k (sec^{-1})	R_m (Ω cm^2)	N_t (10^{-12} mol cm^{-2})
10.11.80	26	0.51	3.0	0.87	0.094	5.8	766	1672	5.1
11.11.80	53	0.85	3.5	0.90	0.170	10.5	337	2406	6.4
12.11.80	35	0.70	3.2	0.90	0.109	17.2	485	3636	7.7
28.11.80	67	1.05	0.1	0.86	0.092	3.9	871	1250	4.3
01.12.80	77	1.01	1.0	0.90	0.094	16.4	560	3450	7.8
16.12.80	59	0.93	2.0	0.90	0.152	12.2	366	2714	6.8
17.12.80	52	0.87	0.07	0.81	0.311	6.0	373	2784	2.5
19.12.80	43	0.69	0.1	0.93	0.197	6.5	249	1276	6.1
23.12.80	46	0.77	2.5	0.90	0.108	11.5	502	2494	7.2
05.01.81	18	0.41	2.5	0.89	0.168	6.5	396	1729	5.1
14.01.81	48	0.81	0.1	0.87	0.102	4.9	726	1451	4.8
15.01.81	52	0.71	0.05	0.87	0.714	5.8	166	2750	1.5
19.01.81	91	1.19	2.0	0.89	0.138	3.9	513	1104	4.4
20.01.81	96	1.01	2.7	0.87	0.121	4.1	643	1277	4.2
22.01.81	102	1.61	0.8	0.88	0.140	4.0	539	1206	4.2
30.03.81	66	0.91	1.6	0.85	0.092	1.2	1169	516	2.3
06.04.81	50	0.63	3.2	0.89	0.126	16.0	464	3744	6.8
14.04.81	50	0.69	0.3	0.93	0.241	3.4	281	924	2.9
16.04.81	109	1.61	1.0	0.89	0.086	2.2	841	637	4.2
21.04.81	74	1.08	1.7	0.87	0.074	0.8	1422	337	2.0
25.05.81	56	0.80	1.2	0.91	0.148	9.8	350	2033	7.1

pH 4 are in keeping with the proposed model. Figure 6 shows an experiment in which the same alga as used in Fig. 3 was transferred for 3 min into seawater of pH 4. The injected charge ($Q=2.8\times10^{-9}$ A sec) was the same as in Fig. 3. The finding that the initial voltage V_m^0 is not changed indicates that the absolute value of the total specific capacitance is not influenced by the low pH value. At pH 4 the time constant of the fast relaxation process increased with time and merged into the long one. Therefore, the voltage decay at pH 4 exhibit only one exponential relaxation instead of the two, as found at pH 8.2.

The time constant for this relaxation was 1.33 msec. With the assumption of a total specific capacitance of 0.5 µF cm^{-2} for the two membranes in series, a value of 2660 Ω cm^2 was calculated for R_m. This figure agrees well with values of the specific resistance R_m derived from current-voltage experiments in which a current pulse of 10-msec duration was injected ($R_m=2630\,\Omega\,\mathrm{cm}^2$). The results suggest that the mobile charges bear a negative sign at pH 8.2 and are neutralized at pH 4. The pH effect on the voltage relaxation in charge-pulse experiments was completely reversible. Fig. 7 shows an experiment in which the same alga as in Figs. 3 and 6 had been returned for 60 min to seawater at a pH value of 8.2. It should be noted that the restoration of the original two relaxation processes required a much longer time than the disappearance of one of the relaxations when the pH of the seawater was lowered from 8.2 to 4. In addition, the time needed

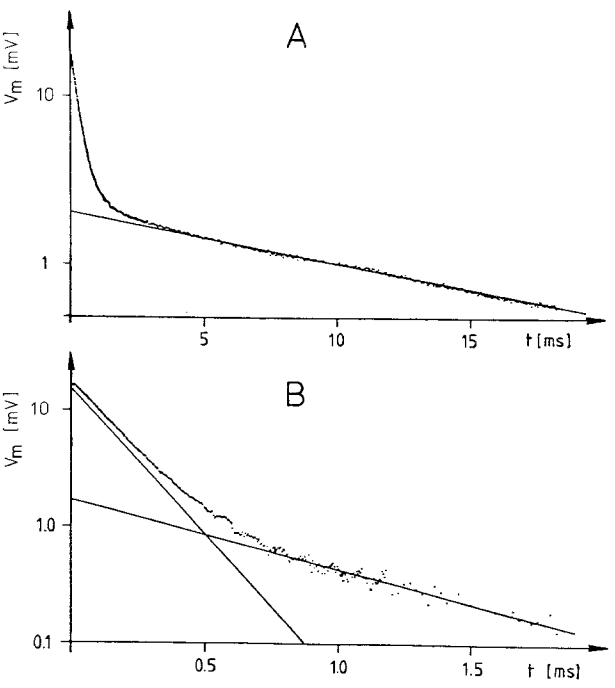

Fig. 5. Semilogarithmic plot of a charge pulse experiment on a *Valonia* cell taken with the digital oscilloscope. The initial voltages $V_{m_1}^0$, $V_{m_2}^0$ and $V_{m_3}^0$ as well as the time constants τ_1, τ_2 and τ_3 were calculated using the method of least squares. (*A*) Semilogarithmic plot of the total relaxation process. (*B*) Semilogarithmic plot of the difference between the data at short times and the fitted third relaxation process (V_{m_3}, τ_3). The inset shows the third fit. $V_{m_1}^0=8.6$ mV; $\tau_1=240$ µsec. $V_{m_2}^0=0.88$ mV; $\tau_2=685$ µsec. $V_{m_3}^0=1.54$ mV; $\tau_3=12.4$ msec. From the data of the first and the third relaxation process the following values for the charge movements were calculated: $k=350$ sec^{-1}; $N_t=4.22\times10^{-12}$ mol cm^{-2}; $R_m=4170\,\Omega\,\mathrm{cm}^2$ ($C_m=0.5$ µF cm^{-2})

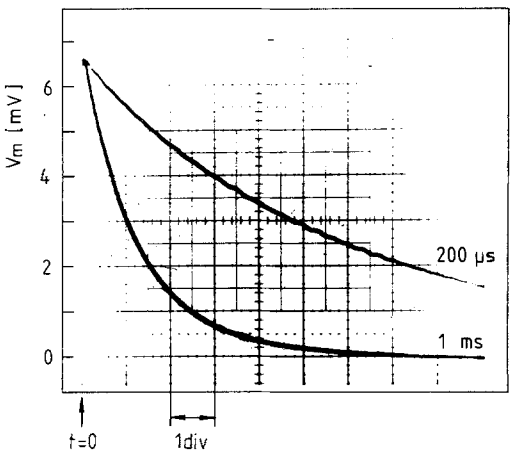

Fig. 6. Oscilloscope record of a charge-pulse experiment on the same cell as shown in Fig. 3, 3 min after changing the pH of the surrounding seawater to pH 4. Pulse duration was 200 nsec, injected charge: 2.8×10^{-9} A sec. In order to calculate the curve better, the voltage relaxation was recorded at two different time resolutions. The fast relaxation has disappeared and both relaxations merge into one by a single exponential decay with a time constant of 1.33 msec. Note that the initial membrane voltage is still about 7 mV, indicating that no change in total membrane capacity has occurred

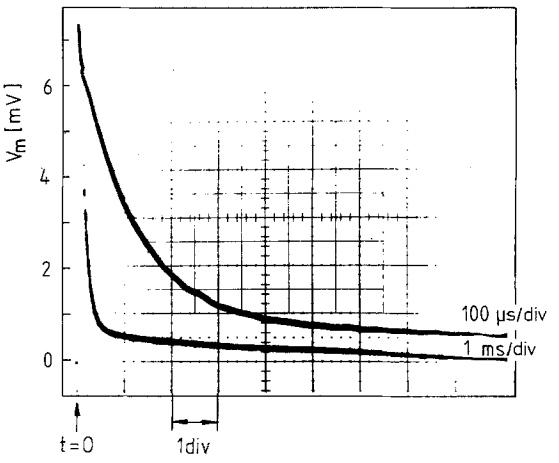

Fig. 7. Voltage-relaxation curve of the same *Valonia* cell as shown in Figs. 3, 4 and 6, 60 min after replacing the seawater of pH 4 by natural seawater of pH 8.2; injected charge 2.8×10^{-9} A sec, 200 nsec duration. Both relaxations which were recorded at different time resolutions appear again with time constants of $\tau_1 = 140\,\mu$sec and $\tau_2 = 5.3$ msec, respectively ($V^0_{m_1} = 5.7$ mV; $V^0_{m_2} = 0.65$ mV). Comparison with the values from Fig. 3 shows that the pH effect is completely reversible ($k = 460\,\text{sec}^{-1}$; $N_t = 5.4 \times 10^{-12}$ mol cm^{-2}; $R_m = 1330\,\Omega\,\text{cm}^2$)

Table 2. Voltage relaxation studies on a cell of *Valonia utricularis* at a pH value of 5 (seawater + 10 mM citrate buffer). At $t = 0$, the pH value of the external seawater was changed

t (min)	a_1	τ_1 (msec)	τ_2 (msec)	k (sec^{-1})	R_m ($\Omega\,\text{cm}^2$)	N_t (10^{-12} mol cm^{-2})
0	0.89	0.094	3.9	717	1047	5.1
1	0.89	0.201	5.2	362	1523	4.2
5	0.89	0.339	9.1	216	2674	4.2
10	0.89	0.488	9.5	160	2952	3.5
15	0.89	0.463	7.6	180	2527	3.1
25	0.87	0.881	6.5	141	3223	1.3
45	0.85	1.809	6.1	111	4905	0.4
60	–	2.6		–	5110	–

for the restoration of the exponential relaxations at pH 8.2 seems to be dependent on the time the cell has been exposed to low pH. If the cells have been bathed for a long time (1–2 hr) in seawater of pH 4, the time for complete recovery of the two relaxation processes at pH 8.2 is at least 10–12 hr.

If the pH of the seawater is lowered to pH 5, some time is needed before the first relaxation merges into the second one. The results of a typical experiment of this type with a *Valonia* cell are given in Table 2. As can be seen from Table 2, N_t as well as k is a function of the time after the pH change. The change of k as a function of pH or time is in principle not consistent with our model. However, a lowering of the pH may change the structure of the membrane, thus resulting in decreasing the translocation rate constant k of the remaining mobile charge.

Further evidence that the two relaxation processes indicate mobile charges within the membrane was obtained from cross-linking experiments with glutardialdehyde. The results of a typical experiment in the presence of glutardialdehyde are given in Table 3. After addition of glutardialdehyde to the seawater a slight decrease in the time constant of the slower relaxation was occasionally observed. In contrast, the time constant of the faster relaxation process shifted towards higher values. At the same time the amplitude ratio of the two processes increases with time. After about 1 hr, only one relaxation was observed with a time constant of 1.20 msec. The resistance R_m which was calculated from the time

Table 3. Voltage relaxation of a cell of *Valonia utricularis* (25 µl) in the presence of 1% glutardialdehyde

t (min)	a_1	τ_1 (msec)	τ_2 (msec)	k (sec^{-1})	R_m (Ωcm^2)	N_t (10^{-12} mol cm^{-2})
0	0.88	0.094	2.1	858	674	3.6
3	0.85	0.097	2.1	986	801	3.1
10	0.83	0.130	2.2	860	986	2.5
20	0.86	0.310	2.1	422	1135	1.2
30	0.57	0.820	1.6	445	2260	0.1
60	–		1.2	–	2400	–

constant of the relaxation by assuming a specific capacity of 0.5 µF cm^{-2}, was 2400 Ωcm^2. This value agrees very well with that obtained by injection of long current pulses into the cell and measuring the corresponding membrane potential difference ($R_m = R_1 + R_2 = 2360\,\Omega cm^2$).

The results can be interpreted in terms of a cross-linkage of the mobile charges. The penetration of glutardialdehyde through membranes should be so fast that the cross-linking reaction presumably occurs in both membranes.

Electrical Breakdown

Reversible electrical-breakdown experiments also support the concept of the presence of mobile charges within the membrane and of nearly identical RC time constants of both membranes. Electrical breakdown was achieved by injecting charge pulses of 800 nsec to 300 µsec duration and high amplitude into the cell (injected charge about 10^{-7} A sec) [30]. After electrical breakdown the slower relaxation component disappeared. Occasionally two breakdown pulses were required for the disappearance of the msec relaxation. The relaxation observed after breakdown had a time constant of 100–140 µsec. After a time interval of 15–60 min the membranes had resealed and the second relaxation was again restored with a time constant in the msec range. This behavior could be explained on the basis of the proposed model. Breakdown leads in general to a large decrease in membrane resistance [1, 28, 30, 35]. Introduction of a low membrane resistance of the order of 200–500 Ωcm^2 into the basic equation of our theory clearly shows that the slow relaxation process speeds up and decreases in amplitude. This leads in the case of very low membrane resistances to only one relaxation. Thus the msec time constant, which is controlled mainly by the resistance of the membrane, decreased and finally disappeared completely. Sometimes, as pointed out previously [30], a splitting of the fast relaxation process into two components after electrical breakdown was observed and might be attributed to the presence of the two-cell membranes, which may have slight different breakdown voltages or resealing times.

Further support for the validity of the proposed model of mobile charges was obtained from measurements of the electrical breakdown voltage of the membrane at pH 4. At pH 8.2 the breakdown voltage measured across the two membranes is on average 0.7 V (when injecting long charge pulses of 300 µsec into the cell) and is increasing to a value of about 2.4 for short charge pulses (0.8 µsec) [30].

As mentioned above, interpretation of the two voltage decays in terms of two RC relaxations leads to membrane capacities of 0.55 and 4.1 µF cm^{-2}, respectively. Thus, when using short charge pulses (800-µsec duration) only one membrane would be charged up to the breakdown voltage, whereas at long pulse lengths the breakdown voltage of both membranes would be reached resulting in the doubling of the apparent breakdown voltage (Holzapfel et al., *in preparation*). This could not be experimentally verified. On the contrary, the opposite relationship was found [30]. Therefore, it can be concluded that the pulse length dependence of the breakdown voltage is an intrinsic property of the membranes [30] and that the specific capacitances of the membranes must be nearly identical.

Furthermore, we found that the breakdown voltage and its pulse-length dependence is pH-independent which is inconsistent with the two-membrane model, but in agreement with the mobile charge concept and with similar experiments performed on artificial lipid bilayer membranes made up of oxidized cholesterol [1].

Our finding that plasmalemma and tonoplast show electrical breakdown contradicts the results of other authors [11] found with cytoplasmotic electrodes in *Chara corallina*. It is accepted, however, that insertion of electrodes into the cytoplasma of plant cells offers many problems which may lead to unreliable potential measurements [23].

Effect of Turgor Pressure

Further evidence that the key assumption of mobile charges is valid comes from measurements of the

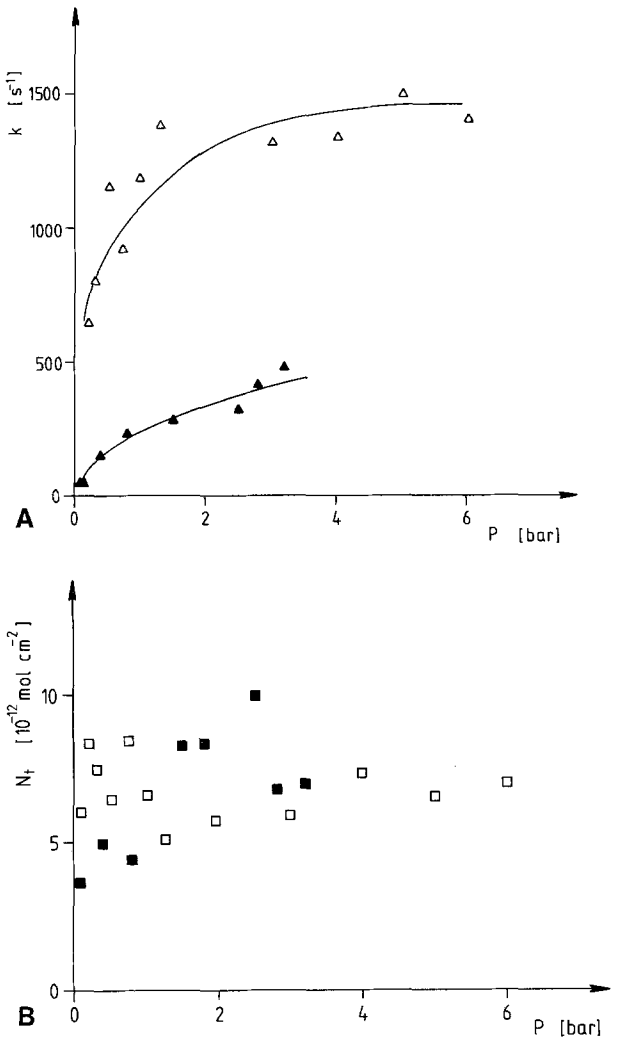

Fig. 8. (*A*) The dependence of the translocation rate k of the mobile charges on turgor pressure for two cells of *Valonia utricularis*. The relationship was calculated from voltage relaxation curves measured at different turgor pressures, using the mobile charge model. It is evident that k is increasing in the pressure interval between 0–2 bar. Cell 1 (15.01.81, Table 4) is denoted by open triangles; cell 2 (06.04.81, Table 4) by filled triangles. (*B*) Pressure dependence of the concentration of the mobile charges N_t in the membranes of *V. utricularis*. Data were derived from the same voltage relaxation curves evaluated in Fig. 8*A*. Whereas the concentration of mobile charges is almost pressure independent in cell 1, the second cell shows an increase in N_t of 100%. (Cell 1 = open squares; cell 2 = filled squares). (*C*) Pressure dependence of the total membrane resistance as calculated from the voltage relaxation curves on the basis of the mobile charge model. Note that the membrane resistance reaches a maximum value at about 2–3 bar in both cells. Cell 1 is denoted by open circles, cell 2 by filled circles. The volumes of both cells were 50 µl

dependence of the voltage relaxation on turgor pressure. The turgor pressure was raised or lowered by adding either distilled water or sodium chloride. It was monitored by the inserted pressure probe. Measurements were performed at water transport

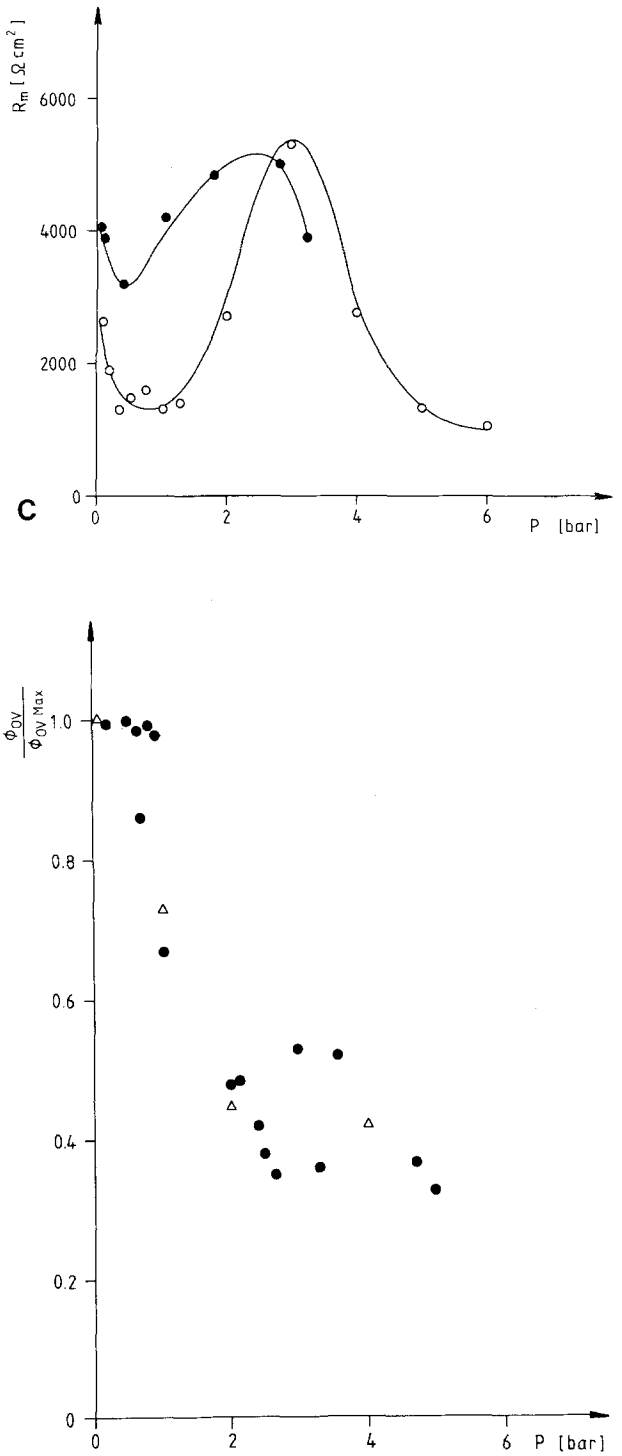

Fig. 9. Pressure dependence of the vacuolar K^+ influx ϕ_{ov} in cells of *Valonia utricularis* over a pressure range of 0 to 4 bar. Due to the variation in the absolute value of the potassium influx from cell to cell, the influx values at a given pressure were related to the maximum K^+ influx at turgor pressure 0.05 bar. It is obvious that the potassium influx decreased markedly from 0 to 2 bar; above 2 bar an almost constant value seems to be reached. Data denoted by a triangle were taken from Steudle et al. [22]. In the volume range of 10 to 100 µl investigated, no volume-dependence of the K^+ influx could be detected

Table 4. Analysis of voltage relaxation curves in terms of the mobile charge model at low and high turgor pressure

Alga	V (µl)	A (cm^2)	P (bar)	a_1	τ_1 (msec)	τ_2 (msec)	k (sec^{-1})	R_m ($\Omega\,cm^2$)	N_t ($10^{-12}\,mol\,cm^{-2}$)
28.11.80	67	1.05	0.1	0.86	0.092	3.9	871	1250	4.3
			2.0	0.83	0.053	4.8	1690	1720	4.1
01.12.80	77	1.01	0.1	0.90	0.094	16.4	560	3450	7.8
			2.0	0.91	0.054	10.3	878	1952	8.7
17.12.80	52	0.87	0.07	0.81	0.311	6.0	373	2784	2.5
			1.8	0.82	0.297	11.2	340	4520	3.3
19.12.80	43	0.69	0.1	0.93	0.197	6.5	249	1276	6.1
			2.0	0.91	0.117	10.9	426	2175	7.8
23.12.80	46	0.77	0.1	0.92	0.088	5.2	543	994	7.5
			2.5	0.90	0.108	11.5	502	2495	7.3
05.01.81	18	0.41	0.1	0.89	0.240	4.8	322	1483	3.6
			2.0	0.90	0.132	8.4	432	1918	6.5
15.01.81	50	0.71	0.05	0.87	0.714	5.8	166	2750	1.5
			2.0	0.88	0.054	4.5	1209	1175	5.8
19.01.81	01	1.19	0.05	0.90	0.110	10.9	496	2378	7.2
			1.6	0.93	0.770	3.5	587	633	7.3
22.01.81	102	1.61	0.05	0.73	0.909	4.5	230	3757	0.7
			1.9	0.86	0.160	4.3	538	1479	3.6
30.03.81	63	0.91	0.04	0.82	0.134	2.8	818	1228	2.7
			1.6	0.85	0.092	1.2	1169	515	2.3
06.04.81	50	0.63	0.02	0.93	1.012	16.6	63	4206	3.5
			1.8	0.91	0.266	24.6	188	4912	7.8
14.04.81	50	0.89	0.02	0.81	2.381	63.2	46	27873	2.8
			1.4	0.88	0.117	16.2	540	4094	6.3
16.04.81	118	1.73	0.02	0.86	0.126	2.7	715	973	3.3
			1.4	0.87	0.069	1.4	1253	484	3.3
21.04.81	77	1.01	0.02	0.87	0.101	4.7	736	1398	4.7
			1.7	0.87	0.074	0.8	1422	337	2.0

equilibrium. The velocity of the turgor pressure change was of the order of 0.5 bar per hr.

Measurements on 14 cells demonstrate that time constants and amplitudes of the two relaxation processes are pressure-dependent. A typical analysis of data in terms of the proposed model presented in Fig. 8 shows that the total surface concentration N_t increased by about 100% from 4 to 8 $\times 10^{-12}\,mol\,cm^{-2}$ in the pressure interval between 0 and 2 bar in one experiment, whereas in the second one N_t was nearly pressure independent. Simultaneously the translocation rate increased from 650 to 1300 sec^{-1} and from 50 to 350 sec^{-1}, respectively (Fig. 8*A* and *B*). In general, the increase in N_t and k with increasing pressure was subject to large variations as indicated in Table 4. In average, k and N_t are at low pressure (0–0.1 bar) 440 sec^{-1} and $4.2 \times 10^{-12}\,mol\,cm^{-2}$ and at higher pressures (1.4–2.5 bar) 800 sec^{-1} and $5.4 \times 10^{-12}\,mol\,cm^{-2}$, respectively.

It should also be noted that the pressure dependence of N_t was sometimes not detected, especially when the turgor pressure of the cells had been changed very rapidly (*see* Fig. 8*B*). On the other hand, only three of the experiments did not exhibit a pronounced pressure-dependence of k. The variation in the data from one experiment to the other is similar to that observed for the pressure-dependence of K^+ influx and efflux. $^{42}K^+$ influx was found to decrease by a factor of 2 to 3 in the pressure interval between 0.05 and about 2 bar, whereas the $^{42}K^+$ efflux increased twofold if the same volume ranges were compared (Figs. 9, 10 and refs. [22, 25]).

Above 1–2 bar the concentration of the mobile charges seemed to reach a constant value. The same was found for the translocation rate, although sometimes a decrease in k was observed when the turgor pressure was further increased. It should be noted that the K^+ influx also became nearly independent of pressure above 2 bar (Fig. 9), whereas the K^+ efflux continued to increase over the whole pressure range (Fig. 10 and refs. [22, 25]. Most importantly, the membrane resistance calculated according to Eq. (19) from the voltage-relaxation process (Fig. 8*C*) exhibited a behavior well-known from measurement of current-voltage relationship as shown in Fig. 11 [36] and showed a maximum value in the pressure range of 3 bar. Due to the opposite pressure-dependence of K^+ influx and K^+ efflux, the membrane resistance increased with turgor pressure up to a maximum value and then decreased with

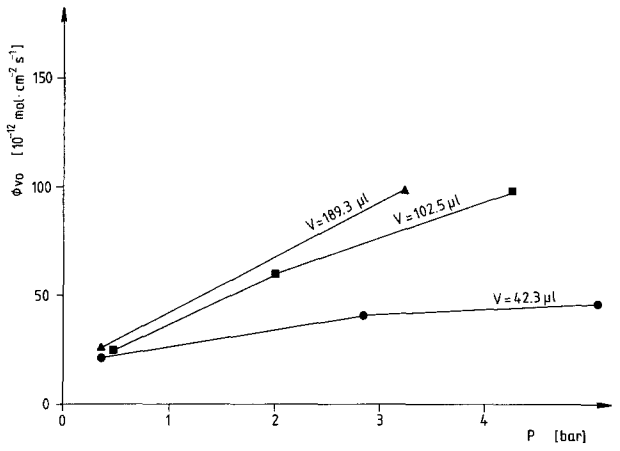

Fig. 10. Pressure dependence of the vacuolar K^+ efflux Φ_{vo} in cells of *Valonia utricularis*. Note, that the efflux increases continuously with increasing turgor pressure. The absolute value for the efflux at a given turgor pressure depends on the cell volume as previously reported [22, 39]

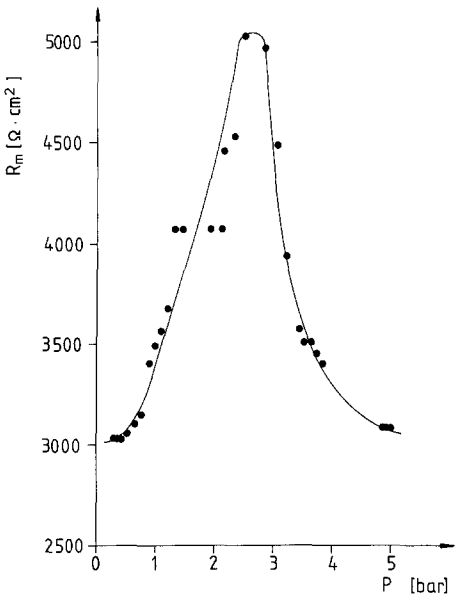

Fig. 11. Effect of cell turgor pressure P on the membrane resistance R_m of a cell of *Valonia utricularis* (cell volume 61 µl). The resistance was calculated from the current-voltage characteristics by using conventional microelectrodes and by injecting constant current pulses of 1 to 5 sec length into the cell. With increasing pressure, the membrane resistance reaches a maximum value and declines then with further increasing pressure. The maximum in membrane resistance occurs in the same turgor pressure range as calculated from the voltage relaxation curves on the basis of the mobile charge model (*see* Fig. 8 *C*)

further increase in turgor pressure. It should be also noted, that in several experiments as indicated in Fig. 8 *C* and in Table 4 an increase in the membrane resistance towards very low pressures was observed. This finding is well-known from membrane resistance measurements using a constant current [36, 39].

Discussion

The results presented here strongly support the view that the two main exponential components in the voltage-relaxation curve of cells of *V. utricularis* arise from mobile charges within the membrane probably involved in a carrier transport system. The interpretation of the two relaxation processes in terms of different time constants of the two membranes (tonoplast and plasmalemma) arranged in series seems to be unlikely for the following reasons. The individual specific capacitances of the two membranes are very unusual and inconsistent with the values reported in the literature for membranes of various species (e.g. [21, 31]). The pulse length dependence of the breakdown voltage [30] and the invariability of the breakdown voltage with pH cannot be explained by the assumption of two membranes (tonoplast and plasmalemma) with different specific capacitances. It has to be noted, however, that for membranes of *Chara corallina* a somewhat higher specific capacitance of both membranes has been found [10]. This higher specific capacitance may be explained on the basis of membrane folding (charasomes [14]), rather than by the assumption of high surface concentrations of mobile charges (Benz, Shimmen & Zimmermann, *unpublished results*). The voltage-relaxation measurements after reversible electrical breakdown of the cell membranes are consistent with the view of mobile charges within the membrane at a concentration of $1\text{–}10 \times 10^{-12}$ mol cm^{-2}. The experiments in which the voltage relaxation was studied in cells fixed with glutardialdehyde can also be reasonably explained in terms of the mobile-charge concept.

Furthermore, the pressure-dependence of the membrane resistance, which was calculated on the basis of Eq. (20) is in agreement with the mobile charge model. The absolute values of the membrane resistance calculated from injecting a constant current are in very good agreement at any given turgor pressure with those values obtained directly by measuring the current-voltage relationship in the conventional way. In addition, both the current-voltage and the charge-pulse technique for measuring membrane resistance showed the same dependence of membrane resistance on pressure: resistance increased with pressure up to a maximum value and then at higher pressure it declined [25, 36, 37, 39].

The pressure-dependence of the membrane resistance was explained by the observation that the

potassium influx decreased with increasing pressure in the pressure interval between 0 and 2 bar; above about 2 bar the potassium influx was nearly pressure-independent [22, 25]. On the other hand, the efflux increased with pressure over the whole pressure range. Furthermore, the magnitude of the efflux at a given turgor pressure is a function of cell size. At the pressure at which the influx and efflux are equal for a given cell size (i.e. the steady state), the membrane resistance is maximum [25, 37]. The small increase in mobile charges within the membrane and the simultaneous strong increase in the translocation rate coincide with the pressure range in which the pressure-dependence of the K^+ influx is pronounced. It is conceivable, therefore, that the slight increase in the mobile charge concentration and the marked increase in the translocation rate are linked to the pressure-dependence of potassium influx (which is an active transport process). The mechanism by which the mobile charges influence the pressure-driven potassium influx or *vice versa* is unclear. It is possible that with increasing pressure a variable number of these charges is set free due to stretching of the membrane and thus interfere with active transport processes. This problem can only be solved if the real nature of these mobile charges is identified. They may be anionic groups attached to proteins.

On the other hand, the increase in the translocation rate and its interference with the pressure-dependent transport processes is more amenable to a theoretical interpretation. We have shown that the translocation rate of dipicrylamine molecules introduced into a planar lipid bilayer membrane increased with decreasing thickness of the lipid bilayer membrane [4]. The thickness of the lipid bilayer membrane could be varied very simply by inclusion of different amounts of solvent within the membrane.

Thinning of the membrane by compression leads to a lowering of the energy barrier in the center of the membrane according to the change in the Born energy w [19]:

$$w=\frac{z^2\cdot e_0^2}{8\pi\varepsilon_0\cdot r}\left(\frac{1}{\varepsilon_m}-\frac{1}{\varepsilon_w}\right)-\frac{z^2\cdot e_0^2}{4\pi\varepsilon_0\,\varepsilon_m\cdot d}\cdot\ln\frac{2\cdot\varepsilon_w}{\varepsilon_w+\varepsilon_m} \tag{26}$$

where e_0 is the charge on the electron, z and r the valency and radius of the ion, respectively. $\varepsilon_0=8.85\times10^{-12}\,\mathrm{Fm}^{-1}$ is the permittivity of free space and $\varepsilon_m=3$ and $\varepsilon_w=80$ are the dielectric constants of the membrane and water. The main contribution to w is the dielectric interaction of the ion with the membrane and the adjacent aqueous phase [19]. For two membranes of dielectric thickness d and d^*, respectively, the difference Δw in the electrostatic energy of an ion located in the central plane of the membrane is given by:

$$\Delta w=w(d^*)-w(d), \tag{27}$$

$$\Delta w=\frac{z^2\cdot e_0^2}{4\pi\varepsilon_0\cdot\varepsilon_m}\cdot\ln\frac{2\cdot\varepsilon_w}{\varepsilon_w+\varepsilon_m}\cdot\left(\frac{1}{d^*}-\frac{1}{d}\right). \tag{28}$$

The translocation rate constant k with respect to the translocation rate k^* at d^* is approximately given by:

$$k=k^*\cdot e^{-\Delta w/KT} \tag{29}$$

where K is the Boltzmann constant and T the absolute temperature (290 °K).

The results presented here show that the translocation rate constant k was approximately doubled when the pressure was raised from 0 to 2 bar. The thickness change of the environment around the mobile charge (presumably a protein) is then 0.4 nm assuming a membrane thickness of 3 nm and a dielectric constant for the membrane material of 3 as calculated from Eqs. (28) and (29). However, the thickness change of the environment of the mobile charge can also be larger if its dielectric constant is higher or if the membrane is thicker at this particular point. The thickness change can be used to calculate the elastic compressive modulus Y_m, according to [25]:

$$\Delta P=Y_m\cdot\ln\frac{d^*}{d} \tag{30}$$

where ΔP is the average pressure range in which k is increased by a factor of about 2.

With the above assumptions a value for Y_m of 14 bar is obtained. It is interesting to note that from electrical breakdown measurements on cells of *V. utricularis* Y_m can be derived if breakdown is interpreted in terms of the electromechanical model [12, 25]. If the same values for the dielectric constant and thickness of the membrane are used as in the calculation given above and if a breakdown voltage of 0.4 V for the single membrane (as found for large pulse lengths) is assumed a value of 13 bar for Y_m is calculated [30]. It does not seem to be reasonable to use the value of breakdown voltage of 1.8 V at very short pulse lengths (nsec range) because at high compression rates it can no longer be assumed that the value for Y_m is constant, i.e. independent of membrane thickness (for proof *see* [33]). The increase in Y_m with decreasing membrane thickness leads then to an increase in breakdown voltage [7, 30]. A similar value for Y_m can be obtained from the experimentally determined pressure-dependence of

the breakdown voltage, if the same values for the dielectric constant, the membrane thickness and the breakdown voltage of a single membrane are used [28]. Without giving any theory, it is immediately evident that precompression of the membrane by increasing turgor pressure should decrease the externally applied membrane potential to reach the breakdown voltage.

The agreement between the Y_m values obtained from changes of the translocation rate constant k of the mobile charges with pressure and from the breakdown experiments could be considered as fortuitous, in that the electrical breakdown may occur in regions of the membrane widely separated from the environment of the mobile charges. On the other hand, in view of the excellent agreement of the values deduced from quite different experimental and theoretical approaches, we conclude that there is a high probability that turgor pressure is sensed by changes in membrane thickness, subsequently followed by changes in membrane transport. The changes in membrane transport can arise either from changes in the intrinsic electric field or by changes in the environment of the active pumps (for example by exclusion of water) or by dipole orientation of water and lipid molecules [33, 35]. Both processes would affect membrane transport. The presence of mobile charges, which were not considered in the theory of the electromechanical model, makes it plausible that other processes are affected in the higher pressure range when the spacing of the mobile charges becomes too small, and that Y_m apparently increases towards higher pressures [33].

The present hypothesis requires that the specific capacitance of the membranes should increase with pressure by about 10% in the pressure range of 0 to 2 bar, if we assume that the membrane area and the dielectric constant are pressure independent.

The predicted increase in the specific capacitance could not be verified because the error involved in the capacitance determination (*see* Eq. (23) and ref. [31]) is also of the order of 10%.

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