Contribution of Electrogenic Ion Transport to Impedance of the Algae *Valonia utricularis* and Artificial Membranes

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ABSTRACT The cell membrane of *Valonia utricularis* contains an electrogenic carrier system for chloride (Wang et al., *Biophys. J.* 59:235–248 (1991)). The electrical impedance of *V. utricularis* was measured in the frequency range between 1 Hz and 50 kHz. The analysis of the impedance spectra from *V. utricularis* and its comparison with equivalent circuit models showed that the transport system created a characteristic contribution to the impedance in the frequency range between 10 Hz and 5 kHz. The fit of the impedance spectra with the formalism derived from the theory of carrier-mediated transport allowed the determination of the kinetic parameters of chloride transport through the cell membrane of *V. utricularis*, and its passive electrical properties. Simultaneous measurements of the kinetic parameters with the charge pulse method demonstrated the equivalence of both experimental approaches with respect to the evaluation of the translocation rate constants of the free and the charged carriers and the total density of carriers within the membrane. Moreover, the impedance spectra of the protonophor-mediated proton transport by FCCP (carbonylcyanide *p*-trifluoromethoxyphenyl-hydrazone) were measured in model membranes. The carrier system made a substantial contribution to the impedance of the artificial membranes. The analysis of the spectra in terms of a simple carrier system (Benz and McLaughlin, 1983, *Biophys. J.* 41:381–398) allowed the evaluation of the kinetic and equilibrium parameters of the FCCP-mediated proton transport. The possible application of the measurement of impedance spectra for the study of biological transport systems is discussed.

INTRODUCTION

Cell membranes contain transport systems to maintain the asymmetry between internal and external ionic environment and for the uptake of substrates into the cell. Many of these transport systems are electrogenic, which means that the transport of the substrate occurs together with that of net charges across the membrane. Examples for these electrogenic transport systems are the H^+ or Na^+ driven cotransport of sugar and amino acids (Slayman and Slayman, 1974; Komor and Tanner, 1976; Felle, 1980; Ullrich, 1979; Kimmich, 1980), ion pumps such as the proton or the chloride pumps (Slayman et al., 1973; Gradmann, 1975, 1989; Shimmen and Tazawa, 1980; Graves and Gutknecht, 1977a, b), and the K^+, Na^+-ATPases (Kaplan, 1985; Fendler et al., 1985). From these systems the electrical properties of the chloride pumps of *Acetabularia mediterranea* and of *Halicystis parvula* (Gradmann, 1975, 1989; Gradmann et al., 1982; Tittor et al., 1983; Graves and Gutknecht, 1977a, b) and of the K^+, Na^+-ATPases (Nakao and Gadsby, 1986; De Weer et al., 1988; Rakowski, 1993) have been studied in detail.

Another well studied electrogenic system is the chloride carrier in the cell membrane of the giant marine alga *Valonia utricularis* (Zimmermann et al., 1982; Benz and Zimmermann, 1983; Büchner et al., 1985; Wang et al., 1991). This carrier system has a high surface density (up to 50 nmol/m^2) in the cell membrane, and it contributes to the apparent specific capacitance of the cell membrane in a manner similar to the chloride pump of *A. mediterranea* (Gradmann, 1975; Tittor et al., 1983). Charge pulse relaxation studies on *V. utricularis* allowed the evaluation of the translocation rate constants of the free and the complexed carriers on the basis of a simple carrier model, the Läuger model (Läuger and Stark, 1970; Läuger, 1972). The potential energy barrier for the movement of the charged form of the carrier through the membrane may be described by a square Nernst-Planck type of barrier (Wang et al., 1993a).

Many studies of ion transport processes have been performed using dc voltage, voltage clamp, and charge pulse methods. Alternating electric fields may also be used for the study of membrane transport phenomena. External electric fields are able to drive electrophoretic and electrogenic ion transport across membranes (Tsong and Astumian, 1988; Robertson and Astumian, 1990a, b, 1991). This means that charges associated with intrinsic membrane proteins as part of transport systems respond to alternating electric fields and may undergo dielectric relaxations caused by field-induced conformation changes within the proteins. This has been demonstrated for the transport processes involved in charge transfer in the Na^+, K^+-ATPases (Serpersu and Tsong, 1984; Liu et al., 1990; Markin et al., 1992).

Transport processes influence also the electrical properties of biological membranes. The chloride carrier in *V. utricularis* and the chloride pump in *A. mediterranea* have been shown to increase the apparent specific capacity of the cell membrane. Similarly, absorbed lipophilic ions increase the specific capacity of artificial and biological membranes (Benz and Conti, 1981; Pickar and Brown, 1983). Furthermore, transport systems are able to increase the specific conductance of lipid bilayer membranes and are probably in

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certain cases responsible for the much higher specific conductance of biological membranes as compared with that of artificial membranes (Läuger et al., 1981). Because the specific capacities and conductances are coupled in these cases to the movement of molecules or parts of it within the membranes, their electrical properties are frequency dependent, which means that the membrane impedance is a function of the frequency.

The measurement of electrical impedance or admittance has been used in the past (see Cole (1972) for an overview) for the study of the electrical properties and ion transport of squid giant axon, epithelia and muscle cells (Valdiosera et al., 1974; Diamond and Machen, 1983; Wills, 1984; Lewis and Alles, 1984; Warncke and Lindemann, 1985; Clausen, 1989; Gordon et al., 1989; Kottra and Frömter, 1990; Fishman, 1992; Asami and Takashima, 1994), plant tissues (Zhang et al., 1990; Colombo and Blumwald, 1992; Zhang and Willison, 1993) and for the study of the chloride pump of *A. mediterranea* (Tittor et al., 1983; Hansen et al., 1983). In squid giant axon and snail neuron the kinetics of the ion channels have been investigated by study of the membrane admittance (impedance) (see Fishman (1992) for an overview). The study of the impedance of plant tissues allowed the evaluation of the resistance and capacitance of the cells and the coupling of cells. The evaluation of the impedance of *A. mediterranea* allowed the study of the kinetic properties of the chloride pump. In other cells investigated so far, the contribution of electrogenic transport systems to the cell impedance is unknown.

We applied the analysis of the membrane impedance to the cell membrane of the giant marine alga *V. utricularis*. The analysis of the results was consistent with charge pulse data obtained from the same cells. The presence of the chloride-transport system in the cell membrane (Wang et al., 1991) leads (in addition to the passive properties of the cell membrane) to characteristic changes of the impedance between 10 Hz and 5 kHz. A decrease in the pH of the external media decreased the contribution of the chloride transport. Measurements of the membrane impedance were also performed on the FCCP-mediated proton transport across artificial lipid bilayer membranes, which has been investigated in detail using voltage clamp and charge pulse experiments (Benz and McLaughlin, 1983). The proton transport contributed also to the membrane impedance. Also in this case it was possible to calculate the kinetic parameters of the transport system from the impedance spectra.

MATERIALS AND METHODS

Culture of *V. utricularis* and experimental conditions

V. utricularis cells were originally collected in the gulf of Naples, Italy, and were kept in natural sea water from the North Sea (supplied by Biomaris, Bremen, Germany) under a 12-h light/dark regime at 17 ± 1°C. Before use the salinity of the North Sea water was adjusted to Mediterranean salinity (1.114 osmol/kg, pH 8.1). Cells of nearly elliptical shape were selected (volume ~60 mm^3, surface area ~0.99 ± 0.27 cm^2). During the experiments the cells were immersed in artificial sea water (ASW) containing 545 mM NaCl, 12 mM KCl, 11 mM $CaCl_2$ and 10 mM $MgCl_2$. If not otherwise stated the pH was adjusted to 8.1 by the addition of 10 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethanesulfonic acid)/NaOH (HEPES/NaOH). For the experiments at pH 6 ASW was buffered with 10 mM 2-(*N*-morpholino)ethanesulfonic acid/NaOH (MES/NaOH). For the measurements at pH 4, 10 mM malic acid/NaOH was added to the ASW. All salts were obtained from Merck (Darmstadt, Germany). The buffers were purchased from Sigma (Taufkirchen, Germany). The experiments were performed at 20°C.

Experimental setup

The algal cells were fixed in a Plexiglas chamber perfused with ASW (CH; see Fig. 1). Two pressure-tight pipette-microelectrodes (outer tip diameter about 30 μm) were positioned in the vacuole of the cell (C), one (ME1) for injection of the charge pulses under charge pulse conditions (position 1 of the switch S in Fig. 1) or for the injection of the sinusoidal current for the impedance measurements (position 2 of the switch S in Fig. 1). It consisted of a 10 μm-thick platinum wire (PE) coated with platinum black to minimize its resistance to 10 kΩ. The other internal electrode (ME2) was used for potential-recording during both types of experiments. It was filled with 3 M KCl and contained a silver/silver chloride wire. The whole electrode had an impedance of about 200 kΩ (Wang et al., 1993b). The voltage relaxation and the sinusoidal voltage across the algal cells in the charge pulse and the impedance measurements was measured with respect to the external reference electrode (RE1) placed in the bulk medium close to the alga surface (3 M KCl agar bridge, Ag/AgCl). The external electrode RE2 (a rectangular steel plate, dimensions 28 × 8 mm) was used for injection of the charge pulses and the sinusoidal current.

The whole setup was shielded against electromagnetic waves by a Faraday cage. The charge pulse experiments were performed as has been described previously in detail (Benz and Zimmermann, 1983, Wang et al., 1991). The impedance measurement were performed as follows. The input sinusoidal signal ($i_m(t) = I_m \sin(\omega t)$) was generated by a function generator (FG in Fig. 1; TE 7702, Toellner, Frankfurt, Germany) and fed into the cell via a 1-MΩ resistor (R1) and the internal current electrode ME1. The frequency was varied in steps of 1, 2, 4, and 10 per frequency decade. The injected sinusoidal current induced across the cell a sinusoidal voltage $v_m(t) = V_m \sin(\omega t + \phi)$. The magnitude of the current was such that the sinusoidal voltage did not exceed a few mV. The sinusoidal voltage and the injected sinusoidal current were measured with a digital two-channel storage oscilloscope (DSO; Explorer 2090, Nicolet, Madison, WI). Measurements were performed between 1 Hz and 50 kHz. The maximum time resolution of the whole electrical system was approximately 50 kHz (Wang et al., 1993b).

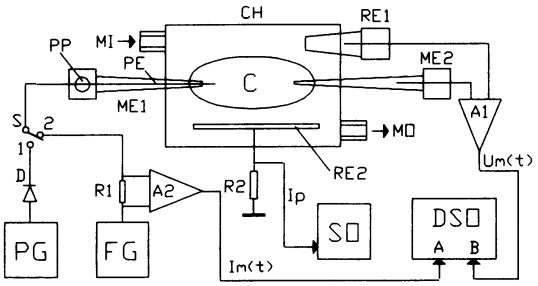

FIGURE 1 Schematic diagram of the setup of the impedance measurements and the charge pulse experiments with *V. utricularis* cells. Abbreviations: A1 and A2, amplifier; C, cell vacuole; CH, Plexiglas chamber; D, the diode with a reverse resistance >10^{10} Ω; DSO, digital two-channel oscilloscope; FG, function generator; ME1, pressure-tight pipette-microelectrode; ME2, pressure-tight pipette-microelectrode; MI and MO, ASW input and output; PE, platinum wire; PG, pulse generator (Model 214B, Hewlett Packard Co., Palo Alto, CA); PP, pressure probe for recording the cell turgor pressure (Zimmermann and Steudle, 1974, 1978); R1, 1-MΩ resistor; R2, 10-Ω resistor; S, switch; SO, storage oscilloscope; RE1, external reference electrode. For further explanations, see text.

The data of current $i_m(t)$ and voltage $v_m(t)$ were transferred in a PC/AT-compatible computer and processed. To calculate the magnitude ($|Z_m| = V_m/I_m$) and the phase angle (ϕ = phase shift between $v_m(t)$ and $i_m(t)$) of the impedance, current and voltage were analyzed by Fast Fourir transformation (FFT). FFT yields real and imaginary parts from which the amplitudes of the current I_m and of the voltage V_m and the phase shift ϕ (by correlation of the two signals) can be calculated. The main advantage of the FFT consisted in the derivation of precise data for $|Z_m|$ and ϕ from the analysis of noisy signals. To analyze the data with respect to the predictions of the transport system the impedance magnitude $|Z_m|$ and phase angle ϕ were plotted against frequency (Bode plots) and fitted with the fit equations given below.

Lipid bilayer experiments

Black lipid bilayer membranes were formed of a 1% solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) dissolved in *n*-chlorodecane (Fluka AG, Buchs, Switzerland). The membranes were spread across circular holes with a diameter of about 1 mm in a wall separating two aqueous phases in a Teflon cell. The aqueous solutions were prepared with 18 MΩ cm water (Millipore Super Q, Millipore Corp., Bedford, MA). They contained 1 M NaCl (analytical grade, Merck, Darmstadt, Germany) and different concentrations of FCCP (carbonyl-*p*-trifluoromethoxyphenylhydrazone; Sigma Chemical Co., Taufkirchen, Germany). The NaCl solution was buffered with 0.1 M KH_2PO_4 and with 0.1 M Tris-HCl and was adjusted to pH 7.4. The experiments were performed at room temperature (20–22°C). The impedance measurements were performed in the same way as described above for the algal cells using a four-electrode system (two voltage and two current silver/silver chloride electrodes).

THEORETICAL BACKGROUND

Impedance of the cell membrane of *V. utricularis*

The mechanism of carrier-mediated transport according to the Läuger model (Läuger and Stark, 1970; Läuger, 1972) has been described in detail in previous publications (Benz and Läuger, 1976; Hladky, 1979; Wang et al., 1991). The interested reader is referred to the review by Läuger et al. (1981) for a complete description. Here only the basic assumptions of the model and the basic equations are listed, which are needed for the calculation of the impedance of carrier-mediated chloride transport in the plasmalemma of *V. utricularis*. The Läuger model assumes a 1:1 carrier-chloride complex, which is formed at the membrane-solution interface. The heterogeneous reaction is described by overall rate constants k_R (association) and k_D (dissociation). The stability constant of the carrier-anion complex is given by $K = k_R/k_D$. In a previous study we have demonstrated that the interfacial reaction is always much faster than all the other reactions involved in carrier-mediated chloride transport. The translocation of the free and charged carriers, k_S and k_{AS}, respectively, are symmetrical and follow first-order kinetics. The translocation rate k_S is rate limiting and the translocation rate k_{AS} shows a voltage dependence of the Nernst-Planck type (Benz and McLaughlin, 1983; Wang et al., 1993a). The translocation of free and charged carriers through the membrane are treated as simple first-order reactions with rate constants k_S and k_{AS}, respectively. The translocation of the charged form of the carrier is the only step within the reaction scheme in which charge is transported across the membrane. This assumption represents a considerable simplification as compared with the treatment of Hladky (1979), in which also the other steps could contribute to the charge transfer across the membrane.

Because the heterogeneous surface reaction is too fast to be resolved in kinetic experiments (Wang et al., 1991, 1993a), the real translocation rate constants for the charged and free carriers k_{AS} and k_S and the stability constant K, for the binding of chloride to the carrier, cannot be obtained from experiments at only one chloride concentration. As shown by Wang et al. (1991, 1993a, b) only the reduced translocation rate constants K_{AS} and K_S of the charged and the free carrier molecules, respectively, can be obtained from the experiments, which are given by

$$K_{AS} = \frac{k_{AS} \cdot K \cdot c}{1 + K \cdot c} \tag{1}$$

and

$$K_S = \frac{k_S}{1 + K \cdot c} \tag{2}$$

Besides the reduced rate constants K_S and K_{AS} the total surface concentration N_0 of the carrier molecules can be calculated from the relaxation parameters (under charge pulse conditions) and the transfer function at a given chloride concentration, c.

The impedance, $Z_m(i\omega)$, of the plasmalemma of *V. utricularis* is given by (Wang et al., 1993b)

$$Z_m(i\omega) = |Z(i\omega)| \cdot \exp(i\phi) = \frac{(i\omega) + a}{C_m((i\omega)^2 + b(i\omega) + c)} \tag{3}$$

with the notations

$$i = \sqrt{-1}$$

and

$$a = 2(K_S + K_{AS}) \tag{4}$$

$$b = 2(K_S + K_{AS}) + 1/(R'_m C_m) + 2BN_0K_{AS} \tag{5}$$

$$c = 2(K_S + K_{AS})\left(\frac{1}{R'_m C_m} + 2BN_0K_{AS}\right) - 4BN_0K_{AS}^2 \tag{6}$$

$$B = \frac{z^2F^2}{4RTC_m} \tag{7}$$

F, R, and T have the usual meaning in Eq. 7; z (= −1) is the valence of the chloride carrier complex. C_m is the specific membrane capacity. G'_m (= $1/R'_m$) is the specific membrane conductance caused by the flux of ions through transport pathways other than the chloride carrier. The total specific membrane resistance R_m is $R_m = Z_m(0)$.

This means that the fit of the membrane impedance as a function of frequency with Eq. 3 allows calculation of the kinetic parameters of carrier-mediated chloride transport (K_S, K_{AS}, and N_0), and of the membrane parameters (C_m and R_m). To test the validity of the fit process we measured the impedance of a model circuit, which simulated the electrical properties of the *V. utricularis* cell (Wang et al., 1993b).

Using different resistors and capacitors within the circuit we were able to calculate them from a fit of the impedance spectra within 5%.

Charge pulse conditions

Under charge pulse conditions the exponential decay of the membrane voltage is given by two exponential relaxations (Wang et al., 1991):

$$V_m(t) = V_0[a_1\exp(-t/\tau_1) + a_2\exp(-t/\tau_2)] \quad (8)$$

$$a_1 + a_2 = 1. \quad (9)$$

From a multiple-exponential-fitting of the voltage decay in the experiments to two exponential relaxations, the initial voltage V_0, the time constants τ_1 and τ_2 and the relative amplitudes a_1 and a_2 could be calculated (Zimmermann et al., 1982; Benz and Zimmermann, 1983; Wang et al., 1991). The specific membrane capacity, C_m, was calculated from

$$C_m = \frac{Q}{V_0 \cdot A} \quad (10)$$

where Q is the injected charge and A the surface area of the alga. The specific membrane resistance R_m and conductance G_m are given by (Benz and Zimmermann, 1983)

$$R_m = \frac{1}{G_m} = \frac{\tau_1 a_1 + \tau_2 a_2}{C_m}. \quad (11)$$

The reduced rate constants of the charged, K_{AS}, and free, K_S, carrier molecules and the total surface concentration of the carrier molecules N_0 can be calculated from the relaxation parameters at a given chloride concentration c (Wang et al., 1991).

$$K_{AS} = (P_1 - P_3 - P_2/P_3)/2 \quad (12)$$

$$K_S = P_2/(2P_3) \quad (13)$$

$$N_0 = P_3/(2BK_{AS}). \quad (14)$$

P_1, P_2, and P_3 are given by the following expressions:

$$P_1 = 1/\tau_1 + 1/\tau_2 \quad (15)$$

$$P_2 = 1/(\tau_1 \cdot \tau_2) \quad (16)$$

$$P_3 = a_1/\tau_1 + a_2/\tau_2. \quad (17)$$

Impedance of the FCCP-mediated proton transport

The mechanism of FCCP-mediated proton transport through lipid bilayer membranes has been described in full detail in a previous publication (Benz and McLaughlin, 1983). It consists also of a Läuger type of ion transport. Here we give only the basic principles of the mechanism and will list the equations needed for the fit of the impedance as a function of the frequency. FCCP is a linear type of protonophor, which means that the membrane current is a linear function of the uncoupler concentration. The binding reaction between anion A^- and proton H^+ takes place at the membrane-solution interface similarly as in the case of the chloride transport in *V. utricularis*. The heterogeneous reaction is described by overall rate constants k_R (association) and k_D (dissociation). The stability constant of the carrier-anion complex is given by $K = k_R/k_D$. In a previous study we have demonstrated that the interfacial reaction is always in equilibrium (Benz and McLaughlin, 1983, Kasianowicz et al., 1987), which means that the FCCP-mediated proton transport is very similar to that of the chloride in *V. utricularis* with the exception that the free carrier (the anion) carries the charge and therefore the current. The translocation of the anion and of the acid are given by the overall rate constants k_A and k_{HA}, respectively, and follows also first-order kinetics.

The impedance, $Z_m(i\omega)$, of a lipid bilayer membrane in the presence of FCCP is given by (see Appendix)

$$Z_m(i\omega) = |Z(i\omega)| \cdot \exp(i\phi) = \frac{(i\omega) + a_0}{C_m((i\omega)^2 + b_1(i\omega) + b_0)} \quad (18)$$

with the notations:

$$a_0 = 2(K_{HA} + K_A) \quad (19)$$

$$b_1 = 2(K_{HA} + K_A) + B_0N_0K_A \quad (20)$$

$$b_0 = 2B_0N_0K_{HA}K_A \quad (21)$$

$$B = \frac{z^2F^2}{4RTC_m}. \quad (22)$$

The translocation rate constants k_A and k_{HA} cannot be derived from experiments at one proton concentration (Benz and McLaughlin, 1983). The reduced rate constants K_A and K_{HA} are given by Eqs. A17 and A18 (see Appendix).

RESULTS

Measurement of the impedance of *V. utricularis*

The impedance measurements of the algal cells *V. utricularis* were started ~1.5–2 h after the impalement with the two internal electrodes. This time was sufficient for complete resealing of the cell membrane around the electrodes, which could be followed by the measurement of the cell turgor. During one single experiment we measured the impedance of the cell at 15 different frequencies between 1 Hz and 50 kHz. Before and after the measurement of the impedance, charge pulse experiments were performed to test whether the electrical properties of the cell had changed. It is noteworthy that the electrical properties of the algal cells showed only insignificant changes during the experiments. Furthermore we followed the cell turgor throughout the whole procedure to make sure that it did not show any substantial change during the time course of an experiment, given that the cell turgor is an important measure of the physiological state of a plant cell.

Analysis of the experimental results

Fig. 2 *A* shows the frequency dependence of the magnitude of the impedance derived from *V. utricularis* cell. The open circles represent the experimental data. The dashed curve A shows the magnitude of the impedance derived from the simplest equivalent circuit model in which the membrane is represented as a parallel resistor-capacitor (R_mC_m) circuit. Curve B represents the frequency dependence of the magnitude of the impedance when a resistor R_z (access resistance) is switched in series with the R_mC_m circuit. It is obvious that curves A and B are not able to explain the experimental data for the magnitude of the impedance of the algal cell. An excellent fit, however, was achieved with curve C ($Z_m(i\omega) + R_z$), which was drawn according to Eq. 3 with the following values for the kinetic constants and N_0: K_{AS} = 580 1/s, K_S = 400 1/s, and N_0 = 5.2 pmol/cm^2. The resistance R_z = 85 Ω was needed to provide a much better fit of the data above a frequency of 5 kHz (curve C). This result probably means that the resistances of the cell interior and exterior including the resistance of the vacuole and the cytoplasm cannot be neglected. The contribution of the stray capacities of the electrodes and the wires could be ignored because they influence the impedance only at very high frequencies (above 100 kHz; impedance of electrodes and wires about 200 kΩ).

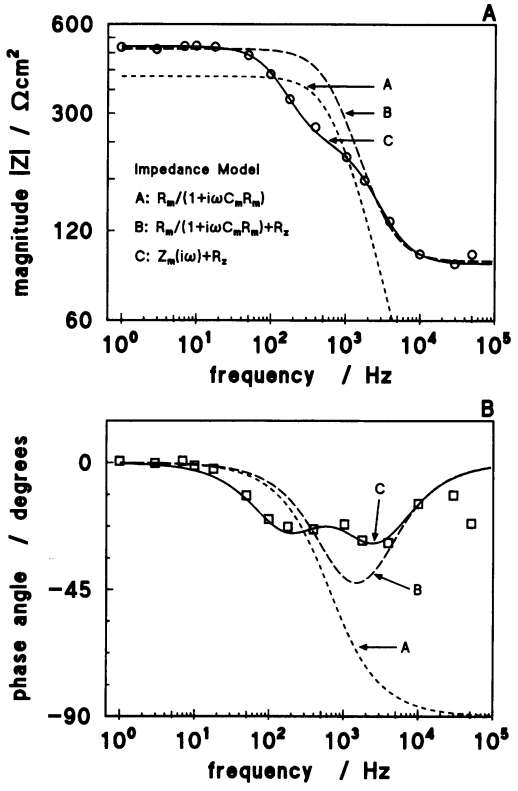

FIGURE 2 Typical frequency dependence of the magnitude (*A*, ○) and the phase angle (*B*, □) of the impedance of a *V. utricularis* cell placed in ASW (pH 8.1); T = 20°C. The surface area A of the cell is 0.98 cm^2. The turgor pressure P (= 0.25 MPa) was approximately constant during the time course of the experiment. The resting membrane potential was 9 mV. The inset shows the theoretical impedance of the different equivalent circuit models for the fit of the data. The impedance $Z_m(i\omega)$ was given by Eqs. 3–7. The curves A, B, and C were fitted by using the corresponding impedance models in the inset, and the following values: R_m = 390 Ω cm^2 is the specific resistance of the cell at very small frequencies (i.e., the stationary resistance). C_m = 0.62 μF/cm^2 was the specific capacity. The total carrier concentration N_0 and the rate constants K_{AS} and K_S were K_{AS} = 580 1/s, K_S = 400 1/s, and N_0 = 5.2 pmol/cm^2. The resistance R_z = 95 Ω was needed to provide a much better fit of the data above a frequency of 5 kHz (curve B). For further explanations, see text.

Similarly, the data for the frequency dependence of the phase angle of the impedance (ϕ) could also not be fitted with simple equivalent circuits (curves A and B), as Fig. 2 *B* clearly shows. Also in this case the best fit was obtained with Eq. 3 (curve C) using the same parameters for K_{AS}, K_S, and N_0, as the corresponding fit of the magnitude of the impedance. This means that the analysis of the impedance spectra of *V. utricularis* cells was not possible on the basis of a simple RC circuit including the impedance of the electrodes and wires.

Table 1 contains the results of the best fit of the impedance data derived from nine *V. utricularis* cells immersed in ASW. The fit was performed as shown in Fig. 2, *A* and *B*, by using Eqs. 3–7. C_m is the passive specific capacity of the cells, and R_m is the specific resistance at very small frequencies (i.e., the stationary resistance). The mean values for the parameters from the nine cells of Table 1 were: N_0 = 5.9 ± 1.1 pmol/cm^2, K_S = 584 ± 234 1/s and K_{AS} = 949 ± 546 1/s. The real translocation rate constants, k_{AS} (= 6230 1/s) and k_S (= 689 1/s) could be calculated from K_{AS} and K_S by assuming K = 0.3 1/M and c = 599 mM (using Eqs. 1 and 2; see also Wang et al. (1991)). It is noteworthy that the values of the specific capacitance C_m, the specific resistance R_m, and the parameters of the chloride carriers as derived from the impedance measurements were in the same range as those that have been obtained previously (Benz and Zimmermann, 1983; Wang et al., 1991, 1993a, b) and from charge pulse experiments performed during this study (see below). The variations of the different parameters of Table 1 from cell to cell were quite normal inasmuch as they probably reflect the physiological state of the cells.

Comparison with the results of charge pulse experiments

The experimental device was organized such that impedance and charge pulse measurement could be performed at the same algal cell. This allowed an efficient control of the fit of the impedance data. Fig. 3 shows a charge pulse experiment performed at the same cell as in Fig. 2. The voltage decay could be explained with two exponential relaxations as has been described previously (Wang et al., 1991). The parameters of these relaxations were: τ_1 = 88 μs, τ_2 = 1.35 ms and a_1 = 0.881 (a_2 = 1 − a_1). The kinetic constants and N_0 were calculated to be: K_S = 416 1/s, K_{AS} = 586 1/s, and N_0 = 5.5 pmol/cm^2. This means that the results of the charge pulse experiments showed excellent agreement with those reported above from the impedance measurements. It is noteworthy that this was generally the case. Table 1 also contains the results obtained from charge pulse experiments

TABLE 1 Results of impedance measurement (IM) and charge-pulse experiments (CPM) performed on nine different *V. utricularis* cells

Experiment (cell no.)	C_m (μF cm^{-2})		R_m (Ω cm^2)		K_{AS} (s^{-1})		K_S (s^{-1})		N_0 (pmol cm^{-2})	
	IM	CPM	IM	CPM	IM	CPM	IM	CPM	IM	CPM
1	0.80	0.77	175	184	816	856	746	755	7.7	7.2
2	0.96	0.99	440	431	1030	1085	198	204	7.2	7.2
3	0.85	0.89	220	189	727	863	842	960	6.1	6.2
4	0.60	0.71	350	303	420	461	612	651	6.0	6.5
5	0.60	0.61	250	224	880	901	668	787	5.5	5.7
6	0.60	0.60	410	380	283	322	765	729	6.2	6.3
7	0.70	0.70	490	423	702	684	272	324	5.4	5.7
8	0.75	0.79	400	329	2170	2772	380	445	4.0	4.2
9	0.60	0.62	420	392	583	598	402	405	5.2	5.5
Mean (± SE)	0.72 (0.13)	0.74 (0.13)	350 (110)	317 (98)	949 (546)	854 (723)	584 (234)	543 (250)	5.9 (1.1)	6.1 (0.9)

The experiments were performed in ASW, pH 8.1; T = 20°C. In the case of (IM), the values of the passive electrical parameters (specific capacitance C_m and specific resistance R_m) of the membrane barrier and of the kinetic parameters (total carrier concentration N_0 and the rate constants K_{AS} and K_S) of the Cl^--transport system were calculated from the optimal curve fitting of the experimentally-determined impedance spectra analogous to those shown in Fig. 2 by using Eqs. 3–7. The results of the charge pulse experiments were determined from plots similar to that shown in Fig. 3 by using Eqs. 8–14.

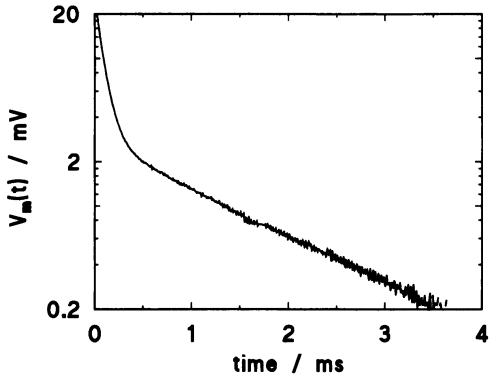

FIGURE 3 Semilogarithmic plot of the voltage versus time of an oscillographic record of a charge pulse experiment performed on the same *V. utricularis* cell as in Fig. 2 bathed in ASW. A charge pulse of 1 μs duration was applied to the cell (injected charge 14 nAs; initial voltage 30 mV). The voltage decay across the cell membranes was fitted to the sum of two different exponential relaxations with the following relaxation parameters: V_1 = 26 mV, V_2 = 4 mV; τ_1 = 88 μs, τ_2 = 1.35 ms. The rate constants calculated according to Eqs. 8–14 were: K_{AS} = 586 1/s; K_S = 416 1/s; N_0 = 5.5 pmol/cm^2; C_m = 0.62 μF/cm^2.

performed on the same nine *V. utricularis* cells. The mean values for the parameters derived from charge pulse experiments of the nine cells of Table 1 were: N_0 = 6.1 ± 0.9 pmol/cm^2, K_S = 543 ± 250 1/s and K_{AS} = 854 ± 723 1/s. The rate constants and N_0 showed satisfactory agreement with those from the fit of the frequency spectra of the magnitude and phase angle of the cell impedance.

Effect of pH on the impedance of *V. utricularis*

We have shown in a previous study that the chloride carrier in *V. utricularis* is influenced by the external pH in such a way that the translocation rate constants decreases with decreasing pH (Benz and Zimmermann, 1983; Wang et al., 1993b). This decrease results in a disappearance of one of the two exponential relaxations under charge pulse conditions (Benz and Zimmermann, 1983), i.e., the plasmalemma exhibits only properties of a simple parallel R_mC_m circuit. To study whether a similar pH effect was observed on the cell impedance, we performed the following experiments. First, we measured the frequency spectrum of the impedance at pH 8.1 (open symbols in Fig. 4, *A* and *B*). The fit of the experimental data was performed using Eq. 3 and the parameters K_{AS} = 450 1/s, K_S = 380 1/s, and N_0 = 5.3 pmol/cm^2. Subsequently, the external pH was lowered to 6, and the frequency spectrum of the impedance was measured again (closed symbols in Fig. 4, *A* and *B*). Both the magnitude and phase angle of the impedance showed substantial changes but it was still possible to fit the experimental data with Eq. 3 (dotted line in Fig. 4, *A* and *B*; K_{AS} = 450 1/s, K_S = 54 1/s, and N_0 = 5.3 pmol/cm^2). Then the cell was taken back to pH 8.1, and the impedance was measured again. After ~30 min the initial values for the parameters of chloride transport were obtained, which means that the pH effect was fully reversible.

The decrease of the pH to 4 resulted in a much larger effect of the pH change on the impedance. In particular, the contribution of the carrier system to the cell impedance decreased so that the membrane impedance could be explained by a fit with $R_m/(1 + i\omega R_mC_m) + R_z$, which was not possible at pH 8.1 (see Fig. 5). Nevertheless, the chloride transporter still had a small influence on the cell impedance as Fig. 5 clearly indicates, and it was possible to derive values for the total carrier concentration N_0 and the rate constants K_{AS} and K_S. When the pH was returned to 8.1, we did not obtain the initial parameters for the carrier system. This result may indicate that the cell was exposed to pH 4 for too long, which may irreversibly alter the carrier sites and influence their kinetics.

Impedance measurements of lipid bilayer membranes

The results described above suggested that chloride carriers contributed to the impedance spectra of the algal cells.

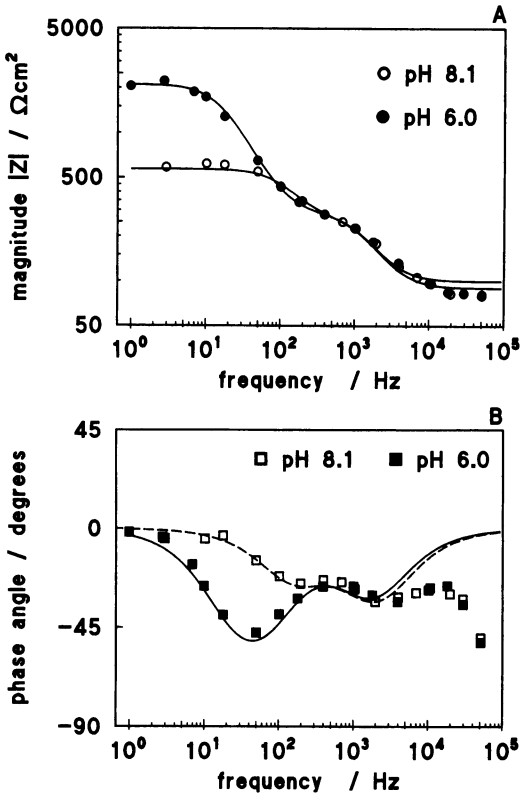

FIGURE 4 Frequency dependence of the magnitude (*A*) and the phase angle (*B*) of the impedance of *V. utricularis* cell W120 at pH 8.1 and pH 6.0. Measurements were performed first at normal pH 8.1 (○), then 30 min after lowering of the pH to 6.0 (●). The curves were fitted by computer by using Eqs. 3–7 and the following values for the total carrier concentration N_0 and the rate constants K_{AS} and K_S: 1) at pH 8.1, K_{AS} = 450 1/s; K_S = 380 1/s; and N_0 = 5.3 pmol/cm²; and 2) at pH 6.0, K_{AS} = 450 1/s; K_S = 54 1/s; and N_0 = 5.3 pmol/cm². Note that at pH 6.0 the contribution of the chloride transporter to the cell impedance decreased in the frequency range <100 Hz. The cell surface was 1.13 cm² and the cell turgor was 0.25 MPa; T = 20°C. The resting membrane potential was 2 mV.

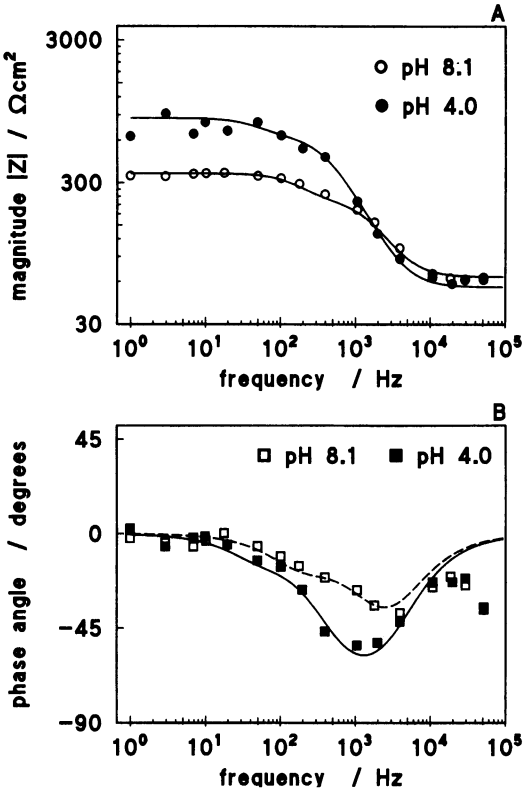

FIGURE 5 Frequency dependence of the magnitude (*A*) and the phase angle (*B*) of the impedance of *V. utricular* is cell W130 at pH 8.1 and pH 4.0. Measurements were performed first at normal pH 8.1 (○), then 30 min after lowering of the pH to 4.0 (●). The curves were fitted by computer by using Eqs. 3–7 and the following values for the total carrier concentration N_0 and the rate constants K_{AS} and K_S: 1) at pH 8.1, K_{AS} = 331 1/s; K_S = 523 1/s; and N_0 = 7.1 pmol/cm²; and 2) at pH 4.0, K_{AS} = 49 1/s; K_S = 152 1/s; and N_0 = 7.1 pmol/cm². Note that at pH 4.0 the contribution of the chloride transporter to the cell impedance decreased in the frequency range <1 kHz. Simultaneously, the membrane resistance R_m increased from 285 to 800 Ω/cm². The cell surface was 0.72 cm², and the cell turgor was 0.33 MPa; T = 20°C.

To study the contribution of carriers to the impedance of a membrane in more detail we performed impedance measurements on artificial lipid bilayer membranes. As a carrier system we used the proton transport by the uncoupler FCCP, which can be explained by a simple carrier system (Benz and McLaughlin, 1983). The experiments were performed at pH 7.4 in the following way. Membranes were formed in the buffered aqueous solutions. After blackening of the membranes the impedance spectrum was measured (see Fig. 6). The impedance of the membrane corresponded to that of a simple parallel RC circuit. Then a concentrated solution of FCCP was added to both sides of the membrane with stirring to allow equilibration, and the impedance spectrum was measured again. Both the magnitude and the phase angle of the impedance were found to be changed, indicating that the transport system had a strong influence on the electrical properties of the membrane. The increase of the FCCP concentration increased the contribution of the carrier system to magnitude and phase angle of the impedance.

The data of Fig. 6 and of similar measurements could be fitted by Eqs. 18–22 by using the parameters given in Table 2. The reduced translocation rate constant, K_{HA}, of the free acid was about 200 1/s, which corresponds to an absolute translocation rate constant, k_{HA} = 4200 1/s, when the surface pKa of FCCP (pK = 6.1) and the proton concentration in the aqueous phase (pH 7.4) are taken into account (Benz and McLaughlin, 1983; see also Eq. A18). The reduced translocation rate constant K_A of the anion was about 380 1/s, which corresponded to k_A = 400 1/s (Eq. A17). At high FCCP concentration the reduced translocation rate constant K_{HA} increased and the partition coefficient $\beta_0 = N_0/2[FCCP]$ decreased somewhat, probably because of saturation effects similar to those that have been observed previously at the same system by using charge pulse and voltage clamp experiments (Benz and McLaughlin, 1983). It is noteworthy that the parameters of FCCP-mediated proton transport derived here from impedance measurement are very similar to those from a previous charge pulse study. This can be seen in Table 2, which also contains the data of charge pulse

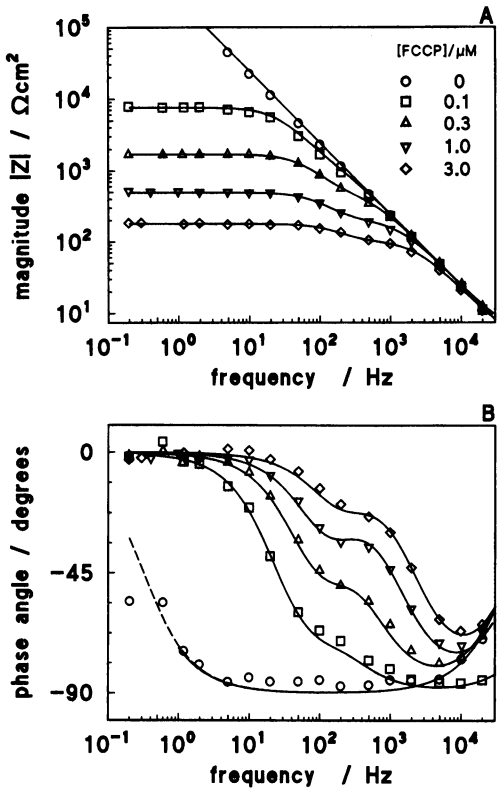

FIGURE 6 Frequency dependence of the magnitude (*A*) and the phase angle (*B*) of the impedance of a lipid bilayer membrane made of diphytanoyl phosphatidylcholine/*n*-chlorodecane. The impedance spectra were measured at five different concentrations of FCCP (0, 0.1, 0.3, 1.0, and 3.0 μM). The aqueous phase contained 1 M NaCl, 0.1 M KH_2PO_4, and 0.1 M Tris-HCl; pH 7.4; the temperature was 20°C. The curves were fitted by computer by using Eqs. 18–22, and the following values for the total protonophor concentration N_0 and the rate constants K_A and K_{HA}: 1) 0.1 μM FCCP, K_{HA} = 202 1/s; K_A = 365 1/s; and N_0 = 0.68 pmol/cm²; 2) 0.3 μM FCCP, K_{HA} = 222 1/s; K_A = 397 1/s; and N_0 = 2.76 pmol/cm²; 3) 1.0 μM FCCP, K_{HA} = 287 1/s; K_A = 371 1/s; and N_0 = 8.45 pmol/cm²; and 4) 3.0 μM FCCP, K_{HA} = 476 1/s; K_A = 340 1/s; and N_0 = 16.8 pmol/cm². Note that the membrane had only passive properties in the absence of FCCP, $R_m > 10^5$ Ω cm² and C_m = 0.83 μF/cm².

experiments taken from Benz and McLaughlin (1983) under similar conditions.

DISCUSSION

The chloride carriers contribute to the impedance of *V. utricularis*

In this study we investigated the effect of the chloride transporter on the cell impedance of *V. utricularis*. Our results suggest that the electrical properties of the cell membrane cannot be described by a simple parallel RC circuit. Similar results have previously been derived from charge pulse and voltage clamp experiments (Wang et al., 1991, 1993a). The contribution of the chloride carrier to the cell impedance is frequency dependent, which means that its magnitude and the phase angle change with the frequency of the input sinusoidal current. The frequency dependence of the magnitude and the phase angle of the impedance could not be explained by time-dependent shifts of the membrane conductance, because the electrical properties of the cells were fairly stable within the time scale of our experiments. Furthermore, we demonstrated in a recent study that the chloride transporter encounters a Nernst-Planck potential barrier and has a very gradual voltage dependence (Wang et al., 1993a), which means that it cannot be responsible for the results described here. The frequency dependence of the impedance is easy to understand, given that the charged groups associated with the carrier system respond to sinusoidal electrical fields only over a limited frequency range (Tsong, 1992). Below that range they contribute to the apparent capacity of the membrane, which results in a change of the impedance spectra. At higher frequencies the carriers cannot follow the field and the membrane has only passive properties.

Effect of pH

The chloride carriers respond to the sinusoidal field within the frequency range between 10 Hz and 5 kHz. A decrease of the external pH to 6 led to a shift of their contribution to the range between 2 and 500 Hz, which means that the velocity of the carriers is decreased. The carrier system has at pH 4 an extremely small contribution to the magnitude and phase angle of the impedance, but this contribution is not negligible, as Figs. 4, *A* and *B* (pH 6), and 5, *A* and *B* (pH 4) clearly indicate. This result was expected, given that the "mobile" charges in the membrane of *V. utricularis* have been found to disappear in charge pulse experiments at low pH (Benz and Zimmermann, 1983). The interesting result of this study was that the small contribution of the carriers at pH 4 had nothing to do with a titration of the negatively charged chloride-carrier complexes themselves as has been discussed previously (Benz and Zimmermann, 1983). Instead, the experimental results of this study provided evidence that the translocation rate constant, K_S, of the free form decreased. Possibly, the carrier protein was immobilized when negatively charged groups were protonated at low pH. On the other hand, we have shown in recent experiments that the ATP level in the cell influences the carrier, i.e., it could be a pump (Wang, Benz, and Zimmermann, unpublished data). This could mean that ATP can no longer bind to the carrier protein because of the protonation of negatively charged groups responsible for ATP-binding.

Advantages of the impedance measurement

The results of the impedance measurements at pH 4 and 6 demonstrate one of the advantages of this method as compared with the charge pulse methods used earlier for the study of the carrier system (Benz and Zimmermann, 1983; Wang et al., 1991). The charge pulse relaxations at pH 4 show only one time constant (Benz and Zimmermann, 1983) and thus reflect only passive properties of the cell membrane. The impedance measurement described here demonstrates that the carrier system contributes at low pH to both the phase angle and the membrane resistance (magnitude) in such a

TABLE 2 Results of impedance measurements on diphytanoyl phosphatidylcholine/*n*-chlorodecane membranes in the presence of different FCCP concentrations

	Impedance technique					Charge pulse technique			
μM FCCP	K_{AH} (10^2 s^{-1})	K_A (10^2 s^{-1})	N_0 (pmol cm^{-2})	β_0 (10^{-3} cm^{-1})	R_m (kΩ cm^2)	K_{AH} (10^2 s^{-1})	K_A (10^2 s^{-1})	N_0 (pmol cm^{-2})	β_0 (10^{-3} cm^{-1})
0.03	2.1	3.6	0.2	3.3	20	2.7 ± 0.1	4.2 ± 0.5	0.32 ± 0.01	5.3
0.1	2.0 ± 0.1	3.8 ± 0.1	0.6 ± 0.1	3.0	6.5 ± 0.5	2.6 ± 0.2	3.8 ± 0.1	1.2 ± 0.1	6.0
0.3	2.1 ± 0.1	3.8 ± 0.3	2.4 ± 0.4	4.0	1.7 ± 0.4	3.1 ± 0.3	4.2 ± 0.3	2.7 ± 0.2	4.5
1.0	2.5 ± 0.2	3.9 ± 0.1	6.7 ± 1.4	3.4	0.54 ± 0.14	3.9 ± 0.3	3.9 ± 0.9	8.3 ± 1.1	4.2
3.0	4.6 ± 0.2	3.6 ± 0.5	14.6 ± 3.1	2.4	0.19 ± 0.03	9.2 ± 1.6	2.1 ± 0.3	23 ± 2	3.8

The aqueous phase contained 1 M NaCl, 0.1 M KH_2PO_4 and 0.1 M Tris-HCl; pH 7.4. The temperature was 20°C. The impedance spectra were fitted by computer by using Eqs. 18–22 and the given values for the total protonophor concentration N_0 and the rate constants K_A and K_{HA} (C_m = 0.83 μF/cm^2). The charge pulse data were taken from Benz and McLaughlin (1983) and are given for comparison.

way that it is possible to derive the kinetic parameters of the transport system. This means that the impedance measurements have a somewhat higher sensitivity toward the detection of the carrier system. The measurement of the impedance also provides a substantial advantage over the harmonic system analysis of *V. utricularis* (Wang et al., 1993b). This is because the harmonic system analysis requires the careful study of the electrodes and the amplifier that is not needed in the impedance measurements.

Does the vacuolar membrane contribute to the cell impedance?

The cytoplasm of a *V. utricularis* cell is very thin (about 10 μm), and it is impossible to insert the electrodes into this cellular compartment. This means that we measured in our experimental approach the impedance of two membrane systems, the cytoplasmic membrane and the vacuolar membrane. Nevertheless, the analysis of our experimental data requires only the assumption of a resistance in series with a single membrane containing the transport system, which was sufficient to explain the impedance spectra up to a frequency of 50 kHz. Within this time range we did not find any indication of the existence of a second parallel RC circuit that could represent the contribution of the vacuolar membrane. This result suggests that the vacuolar membrane has a low resistance, a conclusion that has already been derived from charge pulse and voltage clamp experiments on *V. utricularis*.

A highly conductive tonoplast membrane of *Valonia* disputes the results of Davis (1981) but is consistent with those of Lainson and Field (1976), both derived from *V. ventricosa*. It is noteworthy that isolated vacuoles of various plant cells have a membrane potential and show specific uptake of substrates such as malate or potassium ions when they are energized (Hedrich et al., 1986). Secondly, the specific resistance of isolated vacuoles has recently been measured, and a value of approximately 10^4 Ω cm^2 (similar to the plasmalemma) has been obtained (Bentrup et al., 1986). On the other hand, the vacuole of a marine alga may have a completely different function from that of higher plants, and this function may require a high conductivity for one type of ion but a small salt permeability as we have observed for the vacuole of *V. utricularis* (Wang et al., 1991).

Comparison with other impedance measurements

Impedance measurements have also been performed with *A. mediterranea*, which contains an electrogenic chloride pump (Gradmann, 1975, 1989; Tittor et al., 1983). However, the experimental approach was somewhat different from that used here for the study of *V. utricularis*. Tittor et al. (1983) imposed a steady-state voltage over the sinusoidal current and measured the rate constants as a function of the voltage. The voltage-dependent step of the chloride pump of *Acetabularia* shows a steep dependence on the applied potential, which means that the charged carrier encounters a potential energy of the Eyring type (Tittor et al., 1983). It should be noted, however, that the rate constants of the chloride pump of *Acetabularia* and its voltage dependence have not been measured in direct experiments such as the voltage clamp or the charge pulse methods. The chloride transporter of *V. utricularis* encounters a square barrier, as has been shown by voltage clamp experiments (Wang et al., 1993a).

Impedance spectra have been measured in a number of investigations (see Cole (1972) for an overview) using epithelia (Diamond and Machen, 1983; Wills, 1984; Lewis and Alles, 1984; Warncke and Lindemann, 1985; Clausen, 1989; Gordon et al., 1989; Kottra and Frömter, 1990), human lymphocytes (Bordi et al., 1993), muscle cells (Valdiosera et al., 1974; Asami and Takashima, 1994), squid giant axon (Fishman, 1992), and plant tissues (Zhang et al., 1990; Colombo and Blumwald, 1992; Zhang and Willison, 1993). The results of these measurements are such that the impedance or admittance spectra in some of these investigations cannot be explained by simple RC circuits. Instead they reflect the coupling of cells, the contribution of the transverse tubular system, of ion channel gating and of ion carriers. In particular, in the studies of Zhang et al. (1990) and Clausen and Dixon (1986), the frequency dependence of the impedance of the tissue look quite similar to that shown in Fig. 2, *A* and *B*. This could mean that also in this tissue a transport system contributes to the tissue impedance (Zhang et al., 1990). On the other hand, it is also possible that the electrical

coupling of several different cells results in a disturbance of the impedance spectrum of a simple RC circuit in such a way that several RC circuits are needed to explain the impedance spectra (Diamond and Machen, 1983; Zhang et al., 1990).

Contribution of an electrogenic transport system to the impedance spectra of lipid bilayer membranes

In the experiments on *V. utricularis* cells it was not possible to separate the passive properties of the cell membrane from the contribution of the chloride transporter. Even at pH 4 the transporters contributed to the cell impedance, and its frequency dependence could not be explained by a simple RC circuit. In this study we investigated for the first time the impedance of an artificial lipid bilayer membrane in the presence of a carrier system. These experiments allowed the separation of the RC circuit from the contribution of a transport system to the membrane impedance. This can be seen in Fig. 6. In the absence of FCCP the membrane impedance showed the typical time course of a parallel RC circuit. The addition of increasing concentrations of FCCP resulted in a typical contribution to the spectra of the magnitude and phase angles of the impedance. In particular, within the frequency range between 10 Hz and 5 kHz, the protonophors influenced the phase angle. Increasing the FCCP concentration resulted in a decrease of the membrane resistance and a smaller phase shift (angle), which indicated that the absorbed anions increased the apparent specific capacity of the membrane. Similar conclusions have been derived from charge pulse experiments on squid giant axon in the presence of absorbed lipophilic ions (Benz and Conti, 1981). Similarly, absorbed lipophilic ions contribute also to the apparent specific capacity of lipid bilayer membranes in a certain frequency range (Pickar and Brown, 1983). This has been shown by the measurement of the membrane capacity using the bridge method and sinusoidal voltages of different frequencies (Pickar and Brown, 1983).

APPENDIX

The theoretical description of the FCCP-mediated proton transport has been given in full detail in a previous publication (Benz and McLaughlin, 1983). Here we demonstrate the calculation of the Eqs. 18–22. For this it is assumed that the lipid membrane separates identical proton concentration, c_H. Denoting the interfacial concentrations of the free and complexed FCCP on the left side of the membrane by N'_A and N'_{HA}' and the concentrations on the right side by N''_A and N''_{HA} (expressed in mol/cm^2), then the change of these quantities with time is given by the following four differential equations (Benz and McLaughlin, 1983):

$$\frac{dN'_A}{dt} = -k_R c_H N'_A + k_D N'_{HA} - k'_A N'_A + k''_A N''_A \quad \text{(A1)}$$

$$\frac{dN''_A}{dt} = -k_R c_H N''_A + k_D N''_{HA} + k'_A N'_A - k''_A N''_A \quad \text{(A2)}$$

$$\frac{dN'_{HA}}{dt} = -k_D N'_{HA} + k_R c_H N'_A - k_{HA}(N'_{HA} - N''_{HA}) \quad \text{(A3)}$$

$$\frac{dN''_{HA}}{dt} = -k_D N''_{HA} + k_R c_H N''_A + k_{HA}(N'_{HA} - N''_{HA}). \quad \text{(A4)}$$

It is assumed that the total surface concentration of FCCP, N_0, complexed and free, is constant during a measuring of the impedance spectrum (5 min):

$$N_0 = N'_A + N''_A + N'_{HA} + N''_{HA} = \text{const.} \quad \text{(A5)}$$

Eqs. A1–A4 represent a system of four coupled differential equations. The heterogeneous reaction between H^+ and the anion A^- is always in equilibrium for the FCCP-mediated proton transport ($k_R c_H$, $k_D \gg k_A$, k_{HA}). Accordingly, Eqs. A1-A4 may be reduced to the following single differential equation as has been shown by Benz and McLaughlin (1983):

$$\frac{dx}{dt} = -\frac{1}{(1 + Kc_H)} \cdot [(k'_A + k''_A + 2k_{HA}Kc_H)x + (k'_A - k''_A)r] \quad \text{(A6)}$$

with the notations:

$$r = N'_A + N''_A = \frac{N_0}{1 + Kc_H} \quad \text{(A7)}$$

$$x = N'_A - N''_A. \quad \text{(A8)}$$

The total current density $i_m(t)$ within the lipid membrane has two contributions:

$$i_m(t) = i_p + i_c \quad \text{(A9)}$$

i_c is the current density caused by the movement of anionic FCCP within the membrane:

$$i_c = zF(-k'_A N'_A + k''_A N''_A) \quad \text{(A10)}$$

$z = -1$ is the valence of the anion. i_p is the density of the capacitive current:

$$i_p = C_m \frac{dV_m}{dt} \quad \text{(A11)}$$

C_m is the membrane capacitance. V_m is the membrane voltage. The introduction of Eqs. A10 and A11 into Eq. A9 yields the following relation between current density and membrane voltage when the charge flux is only given by the movement of the anion within the membrane (compare also Eq. 2 of Hladky (1979)):

$$\frac{dV_m}{dt} = \frac{F}{C_m}(-k'_A N'_A + k''_A N''_A) + \frac{i_m}{C_m} \quad \text{(A12)}$$

Of all the rate constants, only k'_A and k''_A are assumed to be voltage dependent. Their dependence is given by a single barrier of the Eyring or Nernst-Planck type (Benz and McLaughlin 1983). For small voltages ($V_m \ll 25$ mV) the expressions may be linearized:

$$k'_A = k_A(1 + u/2) \quad \text{(A13)}$$

$$k''_A = k_A(1 - u/2) \quad \text{(A14)}$$

$u = FV_m/RT$ is the reduced voltage. F, R, and T have the usual meanings. Inserting Eqs. A13 and A14 into Eqs. A6 and A12 yields the following set of two linear differential equations:

$$\frac{dx}{dt} = -2(K_A + K_{HA})x - \frac{FK_A r}{RT} V_m \quad \text{(A15)}$$

$$\frac{dV_m}{dt} = -\frac{F}{C_m}(1 + Kc_H)K_A x - \frac{F^2 N_0 K_A}{2RTC_m} V_m + \frac{i_m}{C_m} \quad \text{(A16)}$$

with the notations:

$$K_A = \frac{1}{(1 + Kc_H)} k_A \quad \text{(A17)}$$

$$K_{HA} = \frac{Kc_H}{(1 + Kc_H)} k_{HA} \quad \text{(A18)}$$

Laplace transformation transfers Eqs. A15 and A16 into a system of two linear differential equations for the complex variables $X(s)$ and $V(s)$, which are the Laplace transformations of $x(t)$ and $V_m(t)$, respectively. $I(s)$ is the

Laplace transformation of $i_m(t)$.

$$sX(s) = -2(K_A + K_{HA})X(s) - \frac{FK_A r}{RT} V(s) \quad \text{(A19)}$$

$$sV(s) = -\frac{F}{C_m}(1 + Kc_H)K_A X(s) - B_0 N_0 K_A V(s) + \frac{1}{C_m} I(s) \quad \text{(A20)}$$

with:

$$B_0 = \frac{F^2}{2RTC_m} \quad \text{(A21)}$$

The elimination of $X(s)$ in Eqs. A19 and A20 allows the calculation of the following equation for $V(s)$ and $I(s)$:

$$V(s) = G(s) \cdot I(s) \quad \text{(A22)}$$

with:

$$G(s) = \frac{V(s)}{I(s)} = \frac{s + a_0}{C_m(s^2 + b_1 s + b_0)} \quad \text{(A23)}$$

with the notations:

$$a_0 = 2(K_{HA} + K_A) \quad \text{(A24)}$$

$$b_1 = 2(K_{HA} + K_A) + B_0 N_0 K_A \quad \text{(A25)}$$

$$b_0 = 2B_0 N_0 K_{AH} K_A \quad \text{(A26)}$$

$G(s)$ is the transfer function of the membrane-carrier system. The impedance of the lipid membrane with the FCCP carrier (Eqs. 18–21) may be obtained by the replacement of the Laplace variable s by $i\omega$ (i.e., $Z_m(i\omega) = G(i\omega)$).

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