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A STUDY OF THE CURRENT-VOLTAGE RELATIONSHIPS OF ELECTROGENIC ACTIVE AND PASSIVE MEMBRANE ELEMENTS IN *RICCIA FLUITANS*

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Potassium- and proton-dependent membrane potential, conductance, and current-voltage characteristics (*I-V* curves) have been measured on rhizoid cells of the liverwort *Riccia fluitans*. The potential difference (E_m) measured with microelectrodes across plasmalemma and tonoplast is depolarized to the potassium-sensitive diffusion potential (E_D) in the presence of 1 mM NaCN, 1 mM NaN_3, or at temperatures below 6°C. Whereas the temperature change from 25°C to 5°C decreases the membrane conductance (g_m) from 0.71 to 0.43 $S \cdot m^{-2}$, 1 mM NaCN increases g_m by about 25%. The membrane displays potassium-controlled rectification which gradually disappears at temperatures below 5°C. The potassium pathway can be described by an equivalent circuit of a diode and an ohmic resistor in parallel. In the potential interval of $E_D \pm 100$ mV the measured *I-V* curves roughly fit the theoretical curves obtained from a modified diode equation. $^{86}Rb^+(K^+)$-influx is voltage sensitive: In the presence of 1 mM NaCN, $^{86}Rb^+$-influx follows a hyperbolic function corresponding to a low conductance at low $[K^+]_o$ and high conductance at high $[K^+]_o$. On the contrary $^{86}Rb^+$-influx is linear with $[K^+]_o$ when pump activity is normal. It is believed that there are two K^+-transport pathways in the *Riccia* membrane, one of which is assigned to the low conductance (0.2 $S \cdot m^{-2}$), the other to a temperature-dependent facilitated diffusion system with a higher conductance (7.7 $S \cdot m^{-2}$). The electrogenic pump essentially acts as a current source and consumes about 39% of the cellular ATP-turnover. In the presence of 30 μM CCCP the saturation current of 0.1 $A \cdot m^{-2}$ is doubled to about 0.2 $A \cdot m^{-2}$, and the electromotive force of −360 mV switches to −250 mV. It is suggested that this may be due to a change in stoichiometry from one to two transported charges per ATP hydrolyzed.

Introduction

Ion transport systems which move net charge across membranes while consuming metabolic energy (i.e. ATP) have been investigated intensively for the past decade in membranes of various kinds of organisms or parts thereof. It is widely accepted now that the genesis of high negative internal potentials (up to −300 mV) by these electrogenic pumps serves as driving belt for the accumulation of ions, or even neutral substrates such as sugars, up to several orders of magnitude inside the cell.

Membranes which can be subjected to electrophysiological analysis by microelectrodes show that, besides this active electrogenic transport, passive electrogenic transport significantly contributes to the electrical behaviour of a cell membrane. By superimposing defined voltage or current steps, the functional relationships between current flow

Symbols and abbreviations: E_m, membrane potential (mV); E_D, diffusion potential (mV); E_i, equilibrium potential of ion j; $g_m = 1/r_m$, electrical membrane (slope) conductance ($S \cdot m^{-2}$); CCCP, carbonyl cyanide-*m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; SHAM, salicylhydroxamic acid.

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through the different membrane pathways and the applied voltage can be analyzed.

Mainly the fungus *Neurospora* [1,2] and giant algae [3–6] have been hitherto subjected to this current-voltage analysis. The present work attempts to characterize electrogenic transport elements by means of *I-V* measurements in rhizoid cells of the aquatic liverwort *Riccia fluitans*; special emphasis is put on the action of inhibitors and temperature in order to separate active and passive pathways: Previous *I-V* analysis of *Riccia* rhizoid cells showed light-induced changes in the *I-V* characteristics [7] and proved a predominance of potassium in the passive electrical membrane properties. Analysis of the passive membrane parameters will therefore be carried out mainly with respect to potassium.

The analysis of the electrogenic pump at the plasmalemma is of great interest because of its importance for the building up and maintenance of gradients across the membrane, and secondly because of its connection to the energy production within the cell. However, the measurement of the *I-V* characteristic of this pump is a difficult task, because the analysis is indirect: (a) the pump mechanism can be inhibited by lowering the ATP-level inside the cell (inhibitors and uncouplers of phosphorylation), or (b) by inactivating the pump itself (low temperature). However, the passive elements cannot be cancelled out experimentally. Therefore particular care is taken to test the reliability of frequently used inhibitors like CN^- or CCCP and their possible direct effect on passive membrane properties.

Materials and Methods

General conditions

Thalli of *R. fluitans* were grown in a 12 h light/dark rhythm in natural pondwater which contained 0.01 – 0.05 mM K^+ and was weakly buffered at pH 8.3. About 48 h before measurements, the tissues were adapted to a test medium with 0.1–10 mM K^+, 20–30 mM Na^+, 10.1 mM Cl^-, 20 mM secondary/tertiary phosphate buffer, and 1 mM Ca^{2+}. In some measurements 1/10 of above concentration was used. The pH was usually kept at 5.5–5.7 and could be varied from 4.5 to 8.4. While changing the ion concentrations, osmolarity was never changed.

CCCP and DNP were predissolved in methanol and stirred into the test solution which was prepared shortly before the experiments. Upon perfusion of the test chamber with the control medium, 90% of the resting potential were regained 3 min after withdrawing CN^-, but 6–10 min were needed after taking CCCP from the medium.

As light source a Leitz-microscopy lamp delivered about 1 $W \cdot m^{-2}$ of white light.

Two morphologically different cells are of electrophysiological interest in *Riccia*: (a) the green thallus cells with mean dimensions of $25 \times 25 \times 60$ μm are suitable for simple potential measurements with one electrode; (b) single opaque cylindrical rhizoid cells with thin cytoplasmic layers and an apical growing tip of 5 to 10 μm. These 2–3 mm long and 20–30 μm wide cells are suitable for conductance measurements with two or three electrodes because of the well defined cable properties (see below).

Electrical measurements

For potential measurements standard glass microelectrode techniques were applied [7]. From glass-fiber containing capillary glass (Hilgenberg) pipettes were pulled on a David Kopf vertical instrument and were backfilled with 0.5 M KCl. Pipettes with tip potentials larger than –25 mV were discarded. The Ag/AgCl-electrodes were connected to a high impedance amplifier (Keithley, 610C or W-P Instruments, M 707). Signals were recorded on a pen-chart or storage oscilloscope. For current-voltage measurements trains of rectangular pulses from a function generator were fed through a resistor (10^{10} Ω) by the current electrode into the midpoint of the rhizoid cell. The voltage response of the membrane was monitored by one or two electrodes at different distances from the current electrode (x_1, V_1, x_2, V_2). The space constant λ was calculated according to

$$\lambda = (x_2 - x_1)/(\ln V_1 - \ln V_2) \quad (1)$$

λ was usually 300 to 400 μm ($\bar{\lambda} = 370$ μm).

Because of $\lambda \ll$ length of rhizoid cell, the conductance data were calculated using the conditions for an infinite linear cable as outlined by Hogg and Williams [8]. Current-voltage curves have been

subjected to the theorem of Cole [9]:

$$i_m = dI_o/dV_o \cdot I_o R_i/4\pi a \quad (2)$$

where dI_o/dV_o is the slope of the input *I-V* curve at V_o and $R_i(\Omega/m)$ denotes the longitudinal resistance of the cell interior; a is the cell diameter.

Extrapolation of I-V curves

For yet unknown reasons, *I-V* curves measured in the presence of metabolic inhibitors or uncouplers, display rapid increase in conductivity at voltages where the control curve appears quite unaffected. In order to estimate roughly the intersection (±20 mV) of two *I-V* curves, extrapolation has been carried out within a short voltage range, according to data from untreated cells.

Regulation of temperature

Experiments were usually carried out at 23 ± 1°C, For experiments at low temperature a thermostatically controlled fluid circuit was used which pumped a H_2O/methanol mixture (15 : 1, v/v) through a plexiglass chamber jacket around the experimental chamber.

Intracellular ATP

After preincubation, green tissue samples of 5–10 mg dry wt. were killed in a mixture of light petroleum/CO_2 (–79°C) and were lyophilized for 48 h. The ATP was extracted with 6% $HClO_4$ at 0°C for 30 min and neutralized with 4 M KOH. After centrifugation at 7000 rev./min for 15 min, in order to discard the $KClO_4$, the supernatant was diluted 1 : 1 and tested for ATP according to Strehler [10] with luciferin/luciferase extract (Sigma) in a bioluminescence equipment (Skan Ag, Basel).

$^{86}Rb^+$-flux measurements

Thalli of *R. fluitans* were allowed to take up $^{86}Rb^+$ from a constantly stirred and thermostatically controlled medium. Specific activity was $2.7 \cdot 10^{12}$ dpm $^{86}Rb^+$ at 1 mM K^+. After 5 min the uptake was stopped and the thalli were rinsed with 150 ml of tracer free medium for 2 min. The samples were frozen and lyophilized for 48 h, weighed and partly dissolved in TS-1 (Zinsser). Unisolve-1 (Zinsser) was added and the samples were tested for activity in a Berthold-Frieseke scintillation counter (BF-5000) for 2 min/sample. Quench corrections for green colour and residue were carried out individually with internal standards ($^{86}Rb^+$).

Results and Discussion

At standard conditions ($[K^+]_o$ = 1 mM, pH_o = 5.6) a potential difference E_m of –200 to –240 mV (inside negative) and an electrical conductance g_m of 0.8 ± 0.22 S · m^{-2} exists across the plasmalemma of *R. fluitans* cells, depending on external pH and $[K^+]_o$. This p.d. was considered too negative to be explained by merely passive electrogenic diffusion ($[K^+]_i \approx 90$–100 mM); therefore an electrogenic pump has been suggested [7].

The equivalent circuit for the membrane potential (inset of Fig. 1) displays two elements in parallel: The pump with an electromotive force E_p and a conductance g_p, and the passive elements (E_D, g_D) which is understood to be the sum of various ion pathways, in *Riccia* being controlled by potassium under the conditions given in Materials and Methods. In order to separate the active from the passive electrogenic elements, three different approaches have been chosen: whereas low temperature should inactivate the pump directly, inhibitors of oxidative phosphorylation (CN^-/SHAM) and protonophores (CCCP, DNP) depress the ATP-level and as a consequence thereof the pump activity.

I. Properties of the pump. The I-V curve

A carrier model for an electrogenic pump by Finkelstein [11] predicts that the pump conductance g_p should be independent of the sign of current passing through the pump, and the highest near its reversal potential. This potential E_p of a charge separating ATPase is simply that voltage at which the net current flow through the pump is zero: For positive outward currents ATP is hydrolyzed, transporting n positive charges through the membrane, whereas for positive inward currents ATP should be synthesized, as it is known from ATPases of mitochondrial or bacterial energy conserving membranes. At large displacements of E_m from E_p, the model postulates a minute conductance and cur-

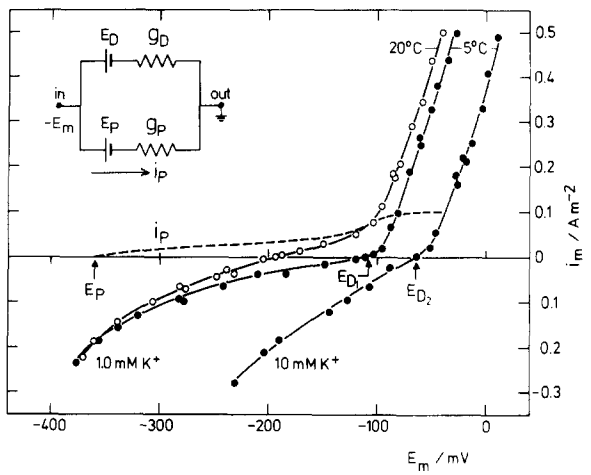

Fig. 1. Steady-state *I-V* curves from *R. fluitans* rhizoid cells at different temperatures and external potassium, as indicated. E_{D1} and E_{D2} denote the membrane potential at 5°C and $[K^+]_o$ = 1 mM and 10 mM, respectively. According to the inset equivalent circuit, subtraction of the 5°C curve (g_D) from the 20°C curve (g_m) yields g_p (i_p, broken line). The intercept with the *V*-axis is then the reversal potential for the pump (E_p).

rent saturation because of the finite number of carrier molecules per unit area of membrane. The equivalent circuit of Fig. 1 yields:

$$g_p = g_m - g_D \tag{3}$$

or

$$i_p = i_m - i_D \tag{3a}$$

and

$$E_p = g_m/g_p \cdot (E_m - E_D) + E_D \tag{4}$$

1. Effects of temperature

As Fig. 1 shows, steady-state *I-V* curves of *Riccia* rhizoid cell membranes are potassium dependent, display a high conductance for positive outward current, and low conductance for inward current [7,12]. These curves increase in curvature at about 0.1 A · m^{-2} at 20°C (Fig. 1; open circles), but near zero current at low temperature (5°C; filled circles), where E_m is depolarized to the diffusion potential (E_{D1}, E_{D2}). This shift of the bend holds also for the *I-V* curves measured in the presence of inhibitors (cf. Fig. 2). The intercept with the *V*-axis is close to the equilibrium potential for potassium, i.e. $E_K \approx E_D$.

According to Eqn. 3, the *I-V* difference curve (i_p, g_p) obtained from the curves at 20°C (g_m) and 5°C (g_D) is assumed to represent the current-voltage curve for the electrogenic pump. This, however, is strictly only true if lowering of the temperature does not significantly change passive parameters. The intersection of the two curves would then define the reversal potential E_p on the *V*-axis which could be measured in *Riccia* within a range of −333 mV to −378 mV (mean −360 mV; Fig. 1; 9 cells).

Unfortunately only few cells survive such strong hyperpolarization without irreversible damage of the plasmalemma (punch-through [13]).

As predicted by the Finkelstein model, the pump *I-V* curve saturates with a low conductance in the neighbourhood of E_D. For *Riccia* rhizoid membranes this saturation current is about 0.1 A · m^{-2}.

2. Effects of CN⁻/SHAM

10^{-3} M NaCN plus 10^{-4} M SHAM [1,12] depolarized the membrane potential to the same level as did

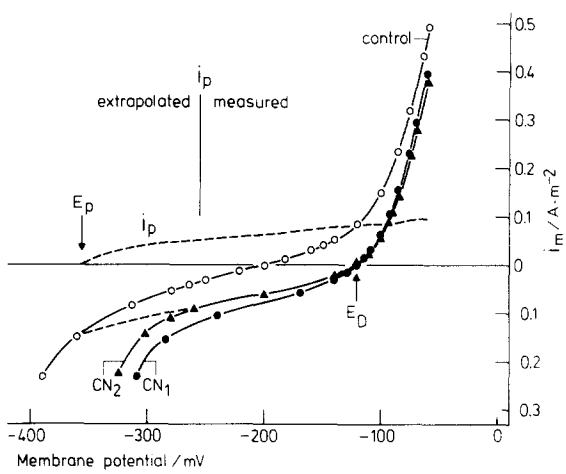

Fig. 2. Effect of 1 mM NaCN + 0.1 mM SHAM upon *I-V* characteristics of *R. fluitans* rhizoid cells. *I-V* control curves were measured shortly before addition of the inhibitors and 30 s after, when membrane depolarization was complete (CN_1). Another curve was taken 1 min after withdrawing CN^-/SHAM from the medium (CN_2). The same subtraction procedure, as outlined in legend of Fig. 1 leads to i_p which partly had to be extrapolated by taking membrane punch-through into account.

lowering of the temperature to 5°C. But whereas low temperature decreased the membrane conductance as one would expect from the equivalent circuit of Fig. 1, i.e. from 0.71 to 0.43 $S \cdot m^{-2}$, NaCN + SHAM induced an increase in conductance by about 25%. The amount of this extra conductance was determined in washout experiments: when CN^- is withdrawn from the external medium there is a time lag before repolarization occurs. Within this time period, which is dependent upon the incubation time with CN^-, the conductance decreases which is attributed to the immediate effect of CN^- upon the plasmalemma: *I-V* curves obtained in the presence of 1 mM CN^- (CN_1) and those measured shortly after washout (CN_2) are shown in Fig. 2. They both intercept the voltage axis at the same membrane potential (E_D), but have different slopes, i.e. conductances. By means of the subtraction method applied in Fig. 1, basically the same difference *I-V* curve (i.e. $g_{control} - g_{CN_2}$) is obtained with a saturation current of 0.1 $A \cdot m^{-2}$ for i_p. Also the extrapolated E_p is in good agreement with the value of −360 mV from the temperature experiment (Fig. 1). Thus the results of both approaches support the idea that the obtained difference *I-V* curves indeed do represent the ATP-hydrolyzing region of the sigmoid pump *I-V* curve.

3. Effects of CCCP

In the presence of 1 μM CCCP, where, for short incubation times, phosphorylation in *Riccia* is not significantly uncoupled [12,14], E_m is depolarized to E'_m and g_m is increased for any current density tested that is from g_m to g'_m (Fig. 3). In the presence of 30 μM CCCP which decreases the ATP level of the tested cells to about 40% (Fig. 4), the *I-V* curves are shifted almost parallel from the g'_m curve to the g'_D curve. g'_D intercepts the voltage axis at $E'_D = -68$ mV which is 43 mV less than the diffusion potential E_D in the presence of 1 mM CN^- (Fig. 2). Change in $[K^+]_o$ from 1 mM (▲) to 0.1 mM (△) now only evokes a minor shift of the *I-V* curves. It has been demonstrated earlier [12] that in the presence of CCCP the *I-V* curves show increased sensitivity to changes in external pH according to the Nernst equation for H^+, i.e. increased proton permeability P_H of the plasmalemma. Subtraction of the g'_D curve taken in the presence of 30 μM CCCP (increased P_H and uncoupled ATP synthesis) from the g'_m curve (only increased P_H) again should display the pump *I-V* curve. But the resulting difference *I-V* curve of Fig. 3 (i'_p) returns a saturation current of roughly 0.2 $A \cdot m^{-2}$, i.e. twice the value obtained in the previous experiments (Fig. 1 and 2).

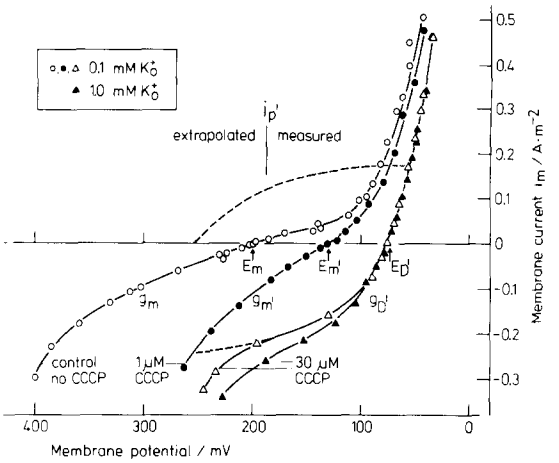

Fig. 3. Effects of different $[K^+]_o$ and CCCP concentrations upon *I-V* characteristics of *R. fluitans* rhizoid cells. As emphasized in legend of Fig. 2, part of the *I-V* difference curve ($g'_m - g'_D$) was extrapolated (punch-through).

II. Stoichiometry of the pump

The free energy ΔG for the ATP-hydrolysis is

$$\Delta G = (E_p + E_H)/nF \qquad (5)$$

where E_H is the equilibrium potential for protons, n is the amount of transported positive charge and F is the Faraday constant. It is possible to calculate the number of charges per ATP split, if E_p and E_H are known. In *Neurospora*, Slayman and coworkers [2] assumed a total of available energy ΔG of 50 $kJ \cdot mol^{-1}$ or −520 mV and demonstrated that the measured E_p of −390 mV and E_H of 40 mV is compatible with a stoichiometry of 1 proton/ATP hydrolyzed. In *Riccia* the situation is less clear: An average of −360 mV for E_p ($E_p$max = −378 mV) and about 50 mV for E_H (pH_i estimated: 6.5–6.8) yields a free energy of only 30 $kJ \cdot mol^{-1}$ for $n = 1$. However it is realistic to assume that the 'true' E_p is

more negative than actually measured because of the punch-through effects due to the strong hyperpolarization of the membrane. On the other hand, the −250 mV for E'_p and the 0.2 A · m^{-2} for i'_p (Fig. 3) suggest a different stoichiometry for the pump in the presence of CCCP. Recent work by Warncke and Slayman on *Neurospora* [15] demonstrates that the doubling of the saturating pump current and the simultaneous halving of the reversal potential may indicate a switch of the pump stoichiometry from one to two transported charges per ATP hydrolyzed. Hence it may well be that in *Riccia,* due to the additional load on the membrane from the CCCP-dependent increased proton permeability, the pump avoids excessive energy loss of the cell by such a change in stoichiometry. Work is in progress to test this preliminary conclusion: Hyperpolarization of the membrane to more negative values than −250 mV in the presence of CCCP should result in ATP synthesis.

III. Voltage source or current source?

The electrogenic pump of *Riccia* is working far from its reversal potential (E_p = −360 mV vs. E_m = −220 mV), and has low conductance compared to the total membrane conductance (g_p = 0.28 S · m^{-2} vs. g_m = 0.7 to 0.8 S · m^{-2}).

Per definitionem, an ideal current source is characterized by an *I-V* curve which is parallel to the voltage axis (i.e. $g = 0$), whereas the *I-V* curve of an ideal voltage source follows $g = \infty$. From Figs. 1 and 2 it is evident that the pump *I-V* curve in *Riccia* is almost parallel to the voltage-axis (=small conductance) in the neighbourhood of E_m, and may therefore rather be referred to as current source than voltage source.

Only very few objects have been analyzed with respect to their pump properties: *Neurospora* also possess this type of electrogenic pump [1,2]. Its reversal potential of −390 mV and the saturation current of 0.1 to 0.2 A · m^{-2} are remarkably close to the values found in *Riccia.* The Cl^--import pump of the giant alga *Acetabularia,* is also understood as a current source, although the membrane potential of −170 mV is close to the reversal potential of −190 mV [5]. The reported saturation current of 0.6 A · m^{-2} differs from the values found for *Riccia* or *Neurospora.*

The characean *Nitella* appears to be an exception: The pump conductance is about 10-times higher than g_D, so that this pump would work as a voltage source, as was concluded by Spanswick [4,6].

IV. Energetics of the cell

Steady state ATP levels are quite stable in the *Riccia* thalli (Fig. 4A, control). However, decay of the ATP cell content is found, when activities of consumers (i.e. the pump) or producers (i.e. phosphorylation) of ATP are altered. The membrane potential already drops at CCCP concentrations of 1 μM or less, where for short incubation times intracellular ATP is not affected significantly [12,14]. In the presence of 30 μM CCCP, E_m drops much faster ($T_{1/2}$ = 3.5 s) than intracellular ATP does ($T_{1/2}$ = 31 s). Equivalent data exist for DNP (not shown). Therefore changes in membrane potential in the presence of low concentrations of these protonophores are understood as a change of proton

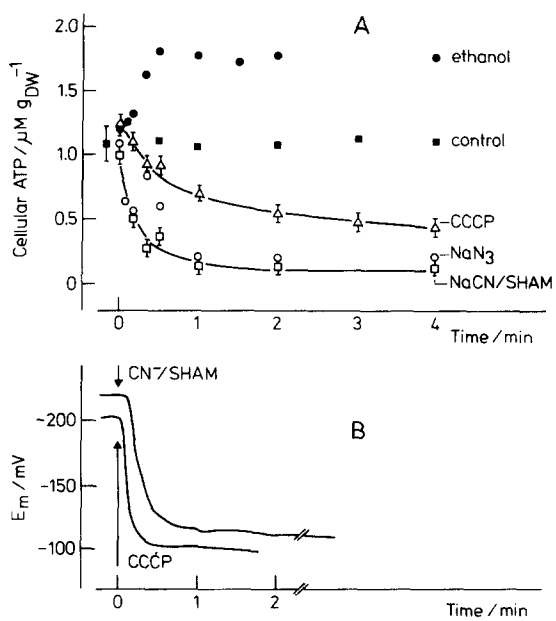

Fig. 4. A. ATP content of *R. fluitans* thallus cells as a function of time in the presence of 30 μM CCCP (▲), 1 mM NaN_3 (○), 1 mM NaCN + 0.1 mM SHAM (□), and 0.1% ethanol (●). B. Depolarization of membrane potential in the presence of 30 μM CCCP and 1 mM NaCN + 0.1 mM SHAM.

permeability, rather than as inhibition of the ATP-dependent electrogenic pump. The membrane potential drops fast ($T_{1/2}$ = 9.3 s, Fig. 4B) and almost parallel with the exponentially fitted ATP decay ($T_{1/2}$ = 11 s; Fig. 4A) in cells where ATP production has been inhibited by $3 \cdot 10^{-3}$ M NaCN plus 10^{-4} M SHAM or 10^{-3} M NaN_3. Similar results have been reported by Slayman et al. [16,17] for *Neurospora*, where depolarization and ATP decay completely correspond in the presence of CN^-, and it has been calculated for *Neurospora* that approx. 30% of the total energy supply is used for transport at the plasmalemma by the H^+-extrusion pump. For *Acetabularia* values up to 50% have been reported by Gradmann [5], for *Griffitsia* however only 0.3% [18].

In *Riccia* the fastest ATP decay was measured in the presence of CN^-/SHAM from which an apparent ATP consumption of $9.24 \cdot 10^{-2}$ W · m^{-2} for the pump was calculated. The power of the pump can be written as

$$N_p = E_p \cdot i_p \qquad (6)$$

where i_p is the saturating current of the pump. From Eqn. 6 a power of $3.6 \cdot 10^{-2}$ W · m^{-2} can be calculated with E_p = −360 mV and i_p = 0.1 A · m^{-2}. This amounts to 39% of the apparent ATP consumption. It seems that the pump in *R. fluitans* is one of the major energy consumers of the cell.

Passive elements of the Riccia membrane

1. K^+ conductance and $^{86}Rb^+$ fluxes

It has been shown for *Riccia* that the K^+ pathway is dominating E_m and g_m over a tested concentration range of 0.1 to 10 mM external K^+ [7]. Because of their small concentration (compared with K^+), protons are less effective, although their permeability appears to be about 10-times higher than P_K [14].

In the presence of 1 mM NaCN and 1 mM $[K^+]_o$, E_m drops from −220 mV (±5 mV) to −111 ± 2 mV, the diffusion potential E_D (42 cells). With an internal concentration of 100 mM this yields

$$E_K = RT/F \ln(0.01) = -116 \text{ mV} \geqslant E_D = -111 \text{ mV} \qquad (7)$$

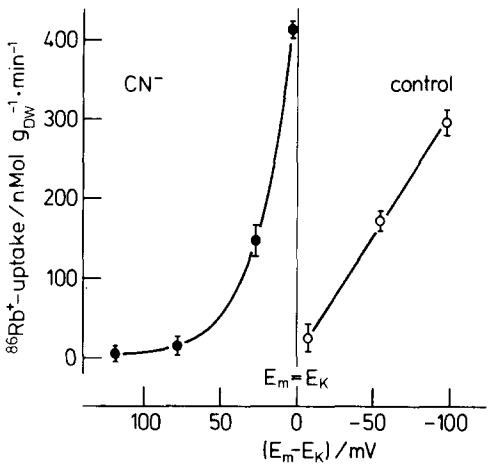

Fig. 5. $^{86}Rb^+$ uptake by *R. fluitans* thallus cells in the presence of 1 mM NaCN (left curve) and without CN^- (control). The uptake data are plotted vs. the difference of E_m (measured) and E_K (calculated). The membrane conductance g_m is represented by the slope ($d\phi Rb^+/dE$) of the curves.

It can be shown that the difference of 3 to 5 mV between E_K and E_D is due to sodium diffusion (P_{Na}/P_K = 0.08) [7].

Using $^{86}Rb^+$ uptake as a measure of independent passive diffusion of K^+, $^{86}Rb^+$ uptake indicates that g_K is constant over a measured range of 2 orders of magnitude of $[K^+]_o$, as long as the pump activity remains unaffected (control curve in Fig. 5). But according to

$$g_K = \phi_K \cdot F/(E_m - E_K) \qquad (8)$$

g_K as a function of $E_m - E_K$ should follow a hyperbolic function. This was found in the presence of 1 mM CN^- where $^{86}Rb^+$ uptake increases drastically as $E_m - E_K$ approaches zero. Hence $^{86}Rb^+$ uptake increases as does g_m, i.e. largely g_K, which suggests that the K^+ pathway now represents almost the total measured membrane conductance.

2. Rectification

The $I-V$ curves from *R. fluitans* rhizoid cells in general display nonlinearity in conductance, i.e. low conductance for positive inwardly directed currents, higher conductance for the opposite. In the absence of the pump activity (CN^-, low temperature), the

characteristic bend is usually very close to zero current (Figs. 1, 2, 6, 7). Phenomena like this have been observed in several genera of plants [13,19,20], in animal cells [9,21,22], have been studied on artificial bilayers [23], and are generally referred to as rectification of the membrane current.

In Fig. 6 it is demonstrated that the membrane changes its rectification properties after cooling from 5°C to 1.5°C. It is possible that the K^+ channel exists in two states and that the in-between curve (3°C) indicates a variable fraction of these two states. At 1.5°C the total $I-V$ curve approximates a straight line with a constant conductance of about 0.4 $S \cdot m^{-2}$.

If it is assumed that these $I-V$ curves mainly represent the potassium pathway, then it can be concluded that this pathway consists of two parallel elements (inset of Fig. 6): (a) one temperature-insensitive system with a low ohmic conductance of 0.4 to 0.5 $S \cdot m^{-2}$ ($i_{1.5°}$), and (b) a system (i_d), possibly facilitated diffusion, with a high conductance of 7.7 $S \cdot m^{-2}$ for K^+ export and a low conductance of 0.2 $S \cdot m^{-2}$ for K^+ import. The sum of both curves results in the measured 5°C $I-V$ curve of Fig. 6.

According to a model, worked out by Mauro [24] and Coster [13], a nonlinearity in the $I-V$ curves can be expected if the cell membrane is composed of two apposed layers of opposite fixed charge, and a potential-dependent Boltzman distribution of free charges exists on either side of the membrane. The swelling and shrinking of a charge depletion layer located between these two fixed charge layers, due to a forward or reverse applied electrical bias, contributes to this rectifying behaviour. The membrane can therefore be regarded within limits as a kind of semiconductor, for which the diode equation [25] should apply. The current i_d through such a system is

$$i_d = I_d \cdot (\exp[qV/kT] - 1) \qquad (9)$$

Large portions of the current-voltage curves measured on *R. fluitans* rhizoid cells in the absence of pump activity can be fitted adequately to this equation or an extension thereof.

In Eqn. 9, k is the Boltzman constant and q denotes the unit of electrical charge $|e|$; T stands for the absolute temperature (K). At 5°C, kT/q =

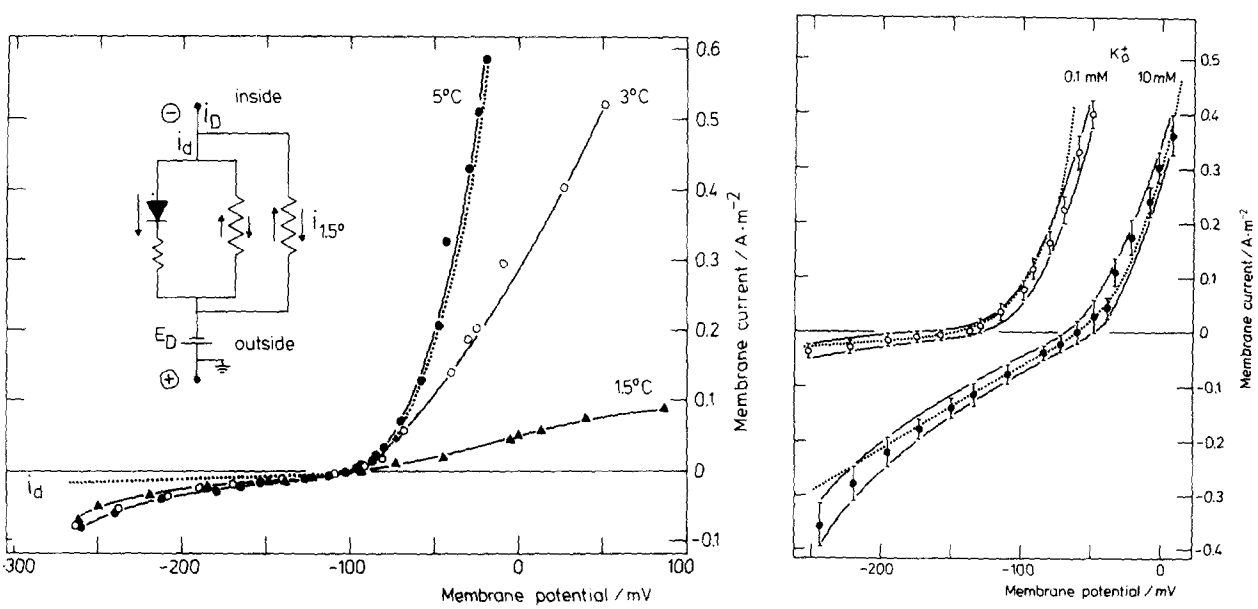

Fig. 6. Effect of temperature changes below 5°C upon I-V characteristics of *R. fluitans* rhizoid cells. According to Eqn. 11, i_d (dotted line) = i_D (•) − $i_{1.5°}$ (▲) (see text).

Fig. 7. I-V curves of *R. fluitans* rhizoid cells at 5°C and external K^+, as indicated. The dotted lines represent the theoretical curves as calculated from the conductance at low membrane current by using Eqn. 11 (see text).

24 mV. V is the displacement of potential to either side of the intercept I-V curve/voltage axis (here E_D) by the injected current: V is positive for membrane depolarizations and negative for membrane hyperpolarizations. I_d is an empirical constant, typical for this plasma membrane; it is sensitive to temperature and has therefore to be adjusted for any temperature change. I_d can be calculated by making $i_d = I_d$ which is the case for $\exp[qV/kT] = 2$. Therefore, at 5°C, $I_d = 2 \cdot 10^{-2}$ A · m^{-2}.

From Fig. 6 it is evident that in the absence of the pump activity the total current through the membrane is

$$i_D = i_d + i_{1.5°} \quad (10)$$

Inserted into Eqn. 9 this yields:

$$i_D = I_d \cdot (\exp[qV/kT] - 1) + i_{1.5°} \quad (11)$$

With Eqn. 11, I-V curves in *Riccia* can be predicted by simply measuring the conductance at E_D. A comparison of measured and calculated curves (dotted lines) is given in Fig. 6 for $[K^+]_o = 1$ mM and in Fig. 7 for $[K^+]_o = 0.1$ mM and 10 mM, respectively. Extreme values were not fitted.

Concluding remarks

It is evident that I-V analysis and the separation of active and passive membrane elements remains inconclusive as long as no specific in-vivo inhibitor of plant plasmamembrane ATPases is available. Even CN^-, often used to inhibit ATP-production, induces additionaly conductance in *R. fluitans* and fails to depress the ATP content of the cells below 25%. This may allow for residual pumping, although the complete depolarization to the diffusion potential raises the question of a threshold ATP concentration available and usable to the pump. Nevertheless, the author is confident that the I-V curves, derived from separate approaches (CN^-, low temperature) are indeed portions of the same curve which saturates around 0.1 A · m^{-2}. This curve probably represents the I-V characteristics of the electrogenic pump in *R. fluitans*.

Although the apparent reversal potential of −360 mV gives no unequivocal information about the amount of charges transported across the membrane per ATP split, a stoichiometry of 1 : 1 appears to be necessary.

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