Simultaneous Measurements of Cytoplasmic K\(^+\) Concentration and the Plasma Membrane Electrical Parameters in Single Membrane Samples of *Chara corallina*

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ABSTRACT

The electrophysiological properties of cytoplasm-rich fragments (single membrane samples) prepared from internodal cells of *Chara corallina* were explored in conjunction with K\(^+\)-sensitive microelectrode and current-voltage (I-V) measurements. This system eliminated the problem of the inaccessible cytoplasmic layer, while preserving many of the electrical characteristics of the intact cells. In 0.1 millimolar external K concentration (K\(_e\)), the resting conductance (membrane conductance \(G_m\), 0.85 ± 0.25 Siemens per square meter (±standard error)) of the single membrane samples, was dominated by the proton pump, as suggested by the response of the near-linear I-V characteristic to changes in external pH. Initial cytoplasmic K\(^+\) activities (a\(_K^+\)), judged most reliable, gave values of 117 ± 67 millimolar; stable a\(_K^+\) values were 77 ± 31 millimolar. Equilibrium potentials for K\(^+\) (Nernst equilibrium potential) (E\(_K^+\)) calculated, using either of these data sets, were near the mean membrane potential (V\(_m\)).

On a cell-to-cell basis, however, E\(_K^+\) was generally negative of V\(_m\); despite an electrogenic contribution from the *Chara* proton pump. When K\(_e\) was increased to 1.0 millimolar or above, G\(_m\) rose (by 8- to 10-fold in 10 millimolar K\(_e\)), the steady state I-V characteristics showed a region of negative slope conductance, and V\(_m\) followed E\(_K^+\). These results confirm previous studies which implicated a K\(_e^+\)-induced and voltage-dependent permeability to K\(^+\) at the *Chara* plasma membrane. They provide an explanation for transitions between apparent K\(_e^+\)-insensitive and K\(_e^+\)-sensitive ('K' electrode') behavior displayed by the membrane potential, as recorded in many algae and higher plant cells.

In the Characeae, as in higher plants, potassium has been thought to be the major permeant ion (see [12, 14] for review). From radioactive tracer flux measurements, the contribution by K\(^+\) to the total membrane ionic permeability was found to be about an order of magnitude greater than the contributions of Na\(^+\) and Cl\(^-\). Adding the expected partial conductances together, however, gave values which were still well below those obtained through direct electrical measurements.

The "missing" conductance has been ascribed to protons, for which no suitable tracer exists. At low K\(_e^+\) (about 0.1 mM)\(^2\)

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\(^{2}\) Abbreviations: SMS, single membrane samples; APW, artificial pond water; K\(^+\), K\(_e^+\), K\(_i^+\), and K\(_\infty^+\) vacuolar, cytoplasmic, internal and external potassium (concentration); a\(_K^+\), (cytoplasmic) potassium activity; E\(_K^+\), Nernst equilibrium potential for potassium; V\(_m\), membrane potential; G\(_m\), membrane conductance; I-V, current-voltage (relation); G-V, conductance-voltage (relation).

membrane potentials recorded were generally more negative than the Nernst equilibrium potentials for any of the major ionic constituents of the cells. This observation provided a first clue to the presence at the plasma membrane of an electrogenic H\(^+\) pump which moved positive charge out of the cell. Metabolic inhibitors abolished the large (negative) V\(_m\) and resulted in potentials which could be accommodated by a combination of diffusion regimes for several monovalent ions (16). Interestingly, K\(^+\) did not appear to contribute significantly to V\(_m\) recorded in submillimolar K\(_e^+\) and in the presence of inhibitors. The estimated E\(_K^+\) was often far negative of V\(_m\). By contrast, the membrane electrical properties underwent a drastic change when K\(_e^+\) was increased above approximately 1.0 mM. (This change, if allowed to happen spontaneously, could take several hours to occur in K\(_e^+\) < 10 mM, but could be induced in these instances by depolarization.) The membrane conductance rose, increasing with K\(_e^+\), and V\(_m\) followed the estimated E\(_K^+\) closely, both in the presence and in the absence of metabolic or ATPase inhibitors.

These observations point to a dichotomy often described both for *Chara* as well as for other algae and higher plants: membrane conductance to K\(^+\) appears to dominate cellular electrical behavior at moderate to high K\(_e^+\), yet at low K\(_e^+\), V\(_m\) is largely independent of E\(_K^+\). Indeed, for *Chara* the transition between K\(_e^+\)-sensitive and K\(_e^+\)-insensitive 'states' is sharp, both temporally and with respect to the extracellular potassium concentration.

Recently, one of us described in *Chara* a K\(_e^+\)-induced conductance which exhibited marked voltage-dependent gating at potentials between about -150 and +50 mV, and which brought the free-running membrane potential close to the expected E\(_K^+\) (5). Such a K\(_e^+\)-dependent feature could go far toward explaining K\(_e^+\)-related properties of plant cell plasma membranes generally, particularly if K\(_e^+\) 'gated' its own conductance. In this paper we address the issue of the conductance identity through simultaneous measurement of a\(_K^+\) and membrane I-V relations. Such experiments have been frustrated in the past by the thin (about 10 \(\mu\)m) cytoplasmic layer, which makes positioning of ion-sensitive and multiple electrodes in the cytoplasm difficult. Since it is the outer membrane, the plasmalemma, which dominates the electrical characteristics of the cell, measurement of K\(_e^+\) is more important than that of K\(_e^+\). One way out of this dilemma is the use of cytoplasm-rich fragments of the giant algal cells. Hirono and Mitsu (13) described a procedure for preparing such fragments—known as 'single membrane samples'—which involved centrifuging and ligating the large internodal cells. While SMS of *Chara* were used previously for obtaining samples of cytoplasm (27), their electrical properties have not been explored. This communication establishes the similarity between SMS and the intact cells and, additionally, presents K\(_e^+\)-selective microelectrode measurements of cytoplasmic a\(_K^+\) from SMS bathed in
K_+^* from 0.1 to 50 mM. We conclude that K^+ does not play an important role in membrane electrical behavior for K_+^* < 1 mM, but for millimolar K_+^* and following transition to the high conductance state, the membrane potential does, indeed, go to E_K, indicating that the conductance must result from a large increase in plasma membrane permeability to K^+.

**MATERIALS AND METHODS**

**Preparation of Single Membrane Samples.** Internodal cells of Chara corallina, which were about 15 cm long and which lacked calcification were selected from between the first and third nodes below the tips of growing plants. Each cell was centrifuged at 1 to 2 g for 30 min, at which time the cytoplasmic plug which gathered at one end of the cell could be observed against a dark background. A silk thread was positioned at the top of the plug (3-5 mm in length) and was loosely tied with a half reef knot. The cell was allowed to lose turgor in the air and the thread was then pulled tight. The rest of the cell was cut off and the remaining SMS was placed in APW (0.1 mM KCl, 1.0 mM NaCl, 0.5 mM CaCl_2, 1.0 mM Hepes adjusted to pH 7.5 with NaOH) to recover. SMS which showed no streaming and/or visible damage were discarded.

While SMS did not survive long enough for cell wall formation, which would have allowed the ligation to be removed (13), they were viable for up to a week and were usually impaled within 2 d of preparing (isolating the internodes in preparing the SMS) did not result in high conductances, as in Nitella axillaris (13). On the contrary, the membrane conductances of the SMS were usually lower than those of the plasmalemmas of intact cells under the same conditions (2). Studies with neutral red dye showed that small vacuoles (1-10 μm in diameter) were present in the streaming cytoplasm of the SMS (G Clint, personal communication). Indeed, all of the normal cytoplasmic (and membranous) constituents could be expected in the preparations. Nonetheless, the term 'single membrane samples' is appropriate in the present context; the SMS lacked a large vacuolar compartment and, for electrical studies, could be treated as a simple cytoplasmic compartment bounded by a single membrane of plasmalammellar origin.

**K^+ Selective Microelectrodes.** The details of the manufacture and use of the K^+-sensitive electrodes have been published previously (8). The glass micropipettes were prepared, silanized, and filled with a small quantity of K^+ ion exchanger (Corning 477317, Corning Medical, Medford, MA). As turgor pressure tended to push the resin up the electrode barrel in early experiments, polyvinylchloride (PVC, cf. [23]) was included at 20% w/v to stabilize the ion exchange resin in the electrode barrel.

Potassium-selective electrodes were back-filled with 1 mM KCl shortly before use and their tips were soaked in 100 mM or 1 mM KCl for about 30 min before calibration. A high input impedance electrometer (FD223, WP Instruments, New Haven, CT) was employed to monitor the potential between the K^+ electrode and a reference. For calibrations the reference was grounded to the bath electrode. For measurements from the SMS, the reference was a second (3 mM KCl-filled) microelectrode implanted in the SMS.

Calibrations were performed in flowing solutions of KCl, 0.1 mM to 1.0 mM, and in mixed solutions with NaCl. Electrodes were chosen which gave >50 mV per decade K_+^* change above 1 mM (Fig. 1). The selectivities were generally 30:1 (K^+ /Na^+) or better, and the electrodes exhibited 90% response times <5 s (equivalent to the time required for solution exchange in the calibration chamber).

**Membrane Potential and Current-Voltage Measurements.** The electrical apparatus has been described previously (6). The cell holder was modified slightly to include the K^+ electrode calibration chamber and to allow access to the cell for insertion of three glass microelectrodes. The SMS were space-clamped by insertion of a Pt/Ir wire through the internode, as in the intact cells. The membrane potential-measuring electrode was inserted before the K^+-sensitive reference electrodes in order to evaluate the viability of the samples. Insertions of the latter two electrodes usually caused only small changes in V_m (Fig. 2A).

To obtain the I-V characteristic of the SMS, the membrane potential was clamped to a bipolar staircase of command voltages and both the current and the potential were recorded every 2 ms under control of a MINC 11 computer (6). The I-V data were fitted with a polynomial and the G-V profile was derived by differentiation.

Where appropriate, data are presented as the mean ± SE.

**Fig. 1.** Potassium microelectrode calibration in 0.1 mM to 1.0 mM KCl. Calibration solution changes indicated by arrows. Inset, calibration curve before cell impalement (●) and after 15 min in the SMS (▲).

**Fig. 2.** A, Typical recording of V_m (upper trace) and a_κ (lower trace) from an SMS exposed to several treatments. SMS impaled with K^+ electrode at time point a. Depolarization with 50 mM K^+^* initially gives rise to a spontaneous action potential at b. Also, the drift to lower a_κ with time. B, Transition to K^+ state in 5 mM K_+^*. The membrane was clamped to 0 mV for about 3 s at time point c to induce the transition. Temporary changes in the a_κ trace during clamping resulted from the slow response of the ion electrode circuit. Spontaneous and large shifts in the trace were remedied by restabbing the reference electrode at time d. I-V scans were taken at times as indicated.
RESULTS

Electrical Characteristics of the SMS. Upon insertion of an electrode into the vacuole of an intact cell, an action potential is usually observed and the cytoplasmic streaming stops. By contrast, when the electrode tip is manipulated into the cytoplasm the voltage signal attains its final value immediately and the streaming continues unabated. The latter behavior was observed when SMS were impaled. A lack of internal compartmentation was indicated, also, by the fact that the electrode could be moved extensively inside the SMS without a change in \( V_m \).

The SMS exhibited the various states found in the intact cells ([4], [7]; Figs. 3, 4). At low \( K^+ \), the \( I-V \) and \( G-V \) profiles displayed a \( pH \) dependence (Fig. 3) similar to that found in the 'pump state' of intact cells (2). The SMS did show lower values for \( G_m \), and \( V_m \) was generally less negative than in the intact cells (Fig. 3; Table 1). In only 2 SMS out of 20 did \( V_m \) achieve or exceed

\(-200 \text{ mV (inside negative)}. \) Action potentials, which are commonly observed in intact cells, were often greatly diminished in magnitude, but near-normal excitation was observed in some samples (M Beilby, unpublished data). Upon exposure to 5.0 mm \( K^+ \) (in APW) the SMS behaved identically to intact cells: the \( V_m \) shifted positive by 20 to 25 mV and then remained unchanged, often for 30 min or more. Clamping \( V_m \) to 0 mV was usually employed to effect the transition into the low-\( V_m \), high-Gm state ('\( K^+ \) state', below), as shown in Fig. 2B. The I-V

\ figure 3. Current-voltage and corresponding conductance-voltage profiles of SMS. Data are for SMS in 0.1 mm \( K^+ \) at \( \text{pH} 4.5 \) (C, ---), \( \text{pH} 7.5 \) (O, ---), and \( \text{pH} 11.0 \) (A, ---). At pH 11 the conductance of intact cells becomes dominated by a large proton permeability (?). The SMS also show this behavior, but the low \( V_m \) and early puncture seen above are not observed in the intact cells.

\ figure 4. \( K^+ \) and the current-voltage characteristics of SMS. Potassium concentrations in the bathing medium were 0.1 mm (O), 2.0 mm (A), 5.0 mm (O), and 10.0 mm (O).

\ figure 5. Current-voltage characteristics of SMS in 50 mm \( K^+ \) (O) and 50 mm \( Na^+ \) (A). Base medium, APW, and salts added as the chlorides. Excitation seemed to disappear in these solutions and was probably a consequence of the lack of an electrochemical gradient for \( Cl^- \). Inset, Comparison of I-V profiles in 0.1 mm \( K^+ \) (C) and 50 mm \( Na^+ \) (A). These records are from a single cell, but data in the inset were gathered on the following day, which accounts for the shift in \( V_m \) for the 50 mm \( Na^+ \) profiles.

\ figure 6. Membrane conductance of SMS as a function of \( K^+ \). Note the log scale on the abscissa. Results from intact cells (O, also gathered in 0.5 mm \( Ca^{2+} \)) were pooled from several studies, and the large standard errors might reflect variability between cultures. \( K^+ \), number of samples (references): 0.1 mm, 17 (2, 4, 5); 2 mm, 4 (3); 5 mm, 7 (3, 4, 5); 10 mm, 21 (4, 5). Results from SMS (O). \( K^+ \), number of samples: 0.1 mm, 6; 2 mm, 5; 5 mm, 7; 10 mm, 4; 50 mm, 3. The single point at 3.5 mm \( K^+ \) was obtained from an I-V scan on an intact cell before it made the transition to the low \( V_m \), high \( G_m \) ('\( K^+ \)') state. Pulses of the I-V staircase were too short to effect the transition. Lines fitted by eye indicate the low membrane conductance associated with the pump and the much larger, \( K^+ \)-dependent conductance observed in the \( K^+ \) state. Intersection of the lines indicates a transition near 0.5 mm \( K^+ \).
characteristic in this state, likewise, compared well with that of
the intact cell (3), although again V_m was slightly more positive
in the SMS (Table I; Fig. 4). Membrane potentials of the SMS
in millimolar K_+ agreed, within experimental error, with E_K
values calculated from the K+-sensitive electrode measurements
(see below).

By contrast with the situation in intact cells (4), it was possible
to voltage clamp SMS over a wide range of potentials (Fig. 5)
without deleterious effects, even in 50 mM K_+ . The I-V profile
displayed all the features typical of that in 5 mM K_+ , but the
curves in the high conductance region of the curve were up to
an order of magnitude greater. These currents were far in excess
of the maximum current usually withstood by the intact cells.
This finding offers an insight into the properties of the tonoplast.
N cascades and large currents passed in intact cells lead to rapid
cellular deterioration (4). In this case, only the plasmalemma
potential is clamped (6); but the current-injecting electrode is
located in the vacuole, so the current necessary to effect the
clamp flows across both plasma membrane and tonoplast. There
are indications that the tonoplast undergoes irreversible damage
if the potential across it becomes too large (19), which might
lead to mixing of the vacuolar contents with the cytoplasm. In
the SMS, current does not pass across the tonoplast and the
plasma membrane is able to withstand large potentials and
currents. (The two membranes in series will survive large currents
and potentials for short times, as was demonstrated in dielectric
breakdown studies [11]).

The conductance of the SMS in >1 mM K_+ calculated from
the I-V curves, are comparable to those of the plasmalemma
in intact cells (Fig. 6). For K_+ from 1.0 to 50 mm, the G_m increased
linearly with the logarithm of K_+ . (Larger conductances than
those indicated in Figure 6 have been obtained in intact cells
after prolonged exposure to 10 mM K_+ [4], but these conduct-
cances may result from cellular damage.) The SMS were also
exposed to 50 mM NaCl APW. As in the intact cells, the K+-
conductance was not observed (4, 15). The I-V profile showed a
marginal increase in G_m over that found in APW alone (1.3 S
m^-2 as opposed to 0.9 S m^-2). Both profiles were roughly linear.
The resting membrane potential in 50 mM Na_+ varied between
-80 and -150 mV (Fig. 5).

**Evaluation of Cytoplasmic a_{K+}** Early measurements of K_+
relied on various macroscopic techniques, such as centrifugation,
for obtaining samples from the major cellular compartments (17,
20, 26, 27). The use of ion-sensitive microelectrodes on giant
algae was pioneered by Vorobiev (28), but has not been repeated
in the Characeae for the last 20 years, apart from recent meas-
urements on isolated cytoplasmic droplets (21).

In the present experiments, the signal from the K+-electrode
was steady, often for >5 min following insertion, and then started
to drift slowly negative (to a lower a_{K+}). This trend continued for
up to 1 h (Table I; Fig. 2). Over this time period V_m did not
change (Table I), and streaming continued apparently unabated.
In fact, impaled SMS survived for many hours. So it was assumed
that the decrease in a_{K+} resulted from the influence of the
cytoplasm on the electrode, rather than vice versa. After 30 min,
the K+-electrode reading occasionally seemed to ‘freeze’ and did
not respond even to drastic measures, such as cutting the SMS
open. On the other hand, when one SMS died shortly after
insertion of the K+-electrode, the a_{K+} recorded in the SMS
decreased over 30 min to the value measured in APW prior to
insertion. Potassium-sensitive electrodes which had been in cells
as long as 30 min could be recalibrated upon withdrawal. Some
loss in response to K_+ was observed on these occasions (Fig. 1,
inset) and it is possible that the electrodes left in the SMS for
longer times were rendered K+-insensitive only by cytoplasm
which gelled in the tip upon exposure to the high Ca_2+-content
of the medium. Nonetheless, the initial measurements must be
judged as more reliable. Since recordings in the various K_+
regimes were made within 10 to 30 min of initial penetration,
these a_{K+} values provide the best estimates for the true values
at these times.

The PVC-containing, K+-electrodes did exhibit high resistances
(50–70 Mohms, roughly 10-fold higher than similar electrodes
prepared without PVC), but electrical noise was not a problem.
The resin was sometimes visibly pushed back on insertion of the
K+-sensitive electrode, despite the presence of the PVC. Bathing
cells in 150 to 200 mM sorbitol to reduce turgor pressure did not
alleviate the problem. Partial blockade of the reference was
another problem which occasionally appeared, especially after
the electrode had been in the cell for >1 h, and which distorted
the readings. Such distortion was usually accompanied by unsta-
able potentials and ‘tracking’ of V_m by the K+-electrode signal.
Withdrawing and reinserting the reference electrode restored the
a_{K+} signal at these times.

A summary of K+-activities recorded and the experimental
conditions are given in Table I. Initial a_{K+} values were obtained
within 2 min of electrode insertion; the stable values were ob-
tained between 10 and 30 min following impalement. The dif-
fences between the two sets of data reflect the drift of the
readings discussed above.

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**Table I. Potassium Activity (a_{K+}) as a Function of External K+ Concentration (K_+)**

<table>
<thead>
<tr>
<th>No. Recordings</th>
<th>No. SMS</th>
<th>K_+</th>
<th>a_{K+}</th>
<th>V_m</th>
<th>E_K</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>12</td>
<td>0.1</td>
<td>117 ± 67</td>
<td>-160 ± 27</td>
<td>-180 (−192, −160)</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>5.0</td>
<td>71 ± 22</td>
<td>-66 ± 13</td>
<td>-70 (−76, −60)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>10.0</td>
<td>70 ± 29</td>
<td>-43 ± 11</td>
<td>-52 (−61, −30)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>50.0</td>
<td>64 ± 11</td>
<td>-23 ± 6</td>
<td>-10 (−14, −5)</td>
</tr>
</tbody>
</table>

*a* Initial values obtained within 5 min of impalement. 
*b* Stable value recorded 10 to 30 min following impalement.

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**Table II. Potassium Activities of Chara Cytoplasm**

Perfusion-aided measurements yielded concentrations, which are
converted to activities using standard activity coefficients (10).

<table>
<thead>
<tr>
<th>a_{K+}</th>
<th>[K+]_e</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>K+ electrode</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>112 Perfusion</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>77 Perfusion</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>K+ electrode in droplets</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>77–117</td>
<td>K+ electrode</td>
<td>This paper</td>
<td></td>
</tr>
</tbody>
</table>

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**Note:** Table II provides a summary of potassium activity measurements in Chara cytoplasm, showing the effect of external potassium concentration on activity coefficients and other relevant parameters.
How do the values in Table I compare to previous measurements? Table II lists the K⁺-electrode data and results from recent perfusion-aided measurements. The initial and stable cytoplasmic aK⁺ span the range obtained by previous workers. (Indirect estimates for K⁺ [24] support cytoplasmic aK⁺ values on the lower side of the range measured. Action potential durations in perfused Chara most closely approximate those of the intact cells when K⁺ was near 100 mM, and Vm was most negative when K⁺ was 65 mM.)

It is of some interest that elevating K⁺ seemed to reduce aK⁺, even when compared to the stable (lower) values obtained in 0.1 mM K⁺ (Table I). This shift is only just visible within the scatter, and is not significant. By contrast, with 50 mM K⁺, the EK values calculated were 7 to 15 mV positive of Vm recorded from 4 out of 5 cells. Under these conditions a K⁺ influx would result, giving an appreciable membrane permeability to K⁺. Nonetheless, changes in aK⁺ upon exposure to high K⁺ must be viewed as artifactual. Assuming that all of the membrane current under voltage clamp is carried by K⁺ and assuming a conductance of 16.2 S m⁻² (Fig. 6), an increase of about 1 μM s⁻¹ could be expected for an SMS with a volume near 3 μl. Such a change would be difficult to detect, even over several hours! It may be possible to detect differences in cells incubated in different K⁺, but such a study will require a careful examination of time-dependent changes in SMS electrical behavior on exposure to high K⁺.)

**DISCUSSION**

Role of Potassium in the Electrophysiology of Chara. Increases in K⁺ (and depolarization by clamping or by spontaneous action potentials) induce the plasmalemma of the intact cells to enter a low Vm, high Gm state (K⁺ state. cf. 13, 15). The membrane potential follows the estimated EK and the I-V characteristic shows a typical negative slope conductance region (3). Under these same conditions, I-V characteristics and conductances of the SMS are very similar to those of intact cells (Fig. 4). The slightly more positive membrane potentials of the SMS may be the consequence of diminished activity of the proton pump, which probably does contribute to the resting potential, even in millimolar concentrations of K⁺ (4, 15). EK values calculated from the activity measurements are close to (within experimental scatter) the Vm data for 2 to 10 mM K⁺ (Table I). At 50 mM K⁺ the potentials agree with the calculated EK only if the initial aK⁺ values (obtained in 0.1 mM K⁺) are used. The implications of this finding are not clear at present, but it is possible that Vm is held more negative than EK through the rising rectifying conductance which is associated with the leakage current (5). The general view that the K⁺ state is dominated by the K⁺ conductance is thus confirmed. This conductance (Fig. 6) rises linearly with log (K⁺), but drops to that seen in low K⁺ at 1 mM. The I-V characteristics in high concentrations of Na⁺ suggest that this K⁺-gated K⁺ conductance (channels?) is highly selective to K⁺ over Na⁺. There are strong indications from radioactive flux and electrical data, that microalgae and higher plants conform to a similar pattern (9, 12, 14, 18).

It has been suggested that the K⁺ conductance in low K⁺ only becomes important if the membrane potential is driven positive by an action potential, by voltage clamping or through the use of inhibitors (cf. 7, 15). Recent experiments (3, 25), however, seem to indicate that the K⁺ channels do not participate in the pump state at all. Results with the SMS seems to confirm this finding. In the SMS the average Vm in the pump state is more positive than EK (the scatter in Table I is high, but out of 14 SMS, 10 showed potentials more positive than the calculated EK), even if the lower stable aK⁺ is taken to be correct. While the proton pump is not as active as in the intact cells, the electrical characteristics of the SMS in low K⁺ are very similar to those of the intact cells. The I-V and G-V profiles show the typical pH dependence (compare Fig. 3 with the results of [2]). The membrane potential and conductance are only weakly dependent on changes in K⁺ (Figs. 2b, 6) and insensitive to tetraethylammonium (inhibits, a K⁺-channel antagonist which blocks the Chara K⁺ state. These facts support the view that the K⁺ channels are closed in low K⁺ (4, 25). The membrane conductance is distributed between the proton pump and a relatively K⁺-insensitive leak pathway across the membrane. It is noteworthy, too, that estimates for Na⁺ of 5 mM (22) indicate that ENa lies near +55 mV, far positive of the Vm in 50 mM Na⁺. Thus, Na⁺, likewise, does not appear to play an important role in the electrical behavior of the plasma membrane.

Finally, the conservation of the K⁺ state in the SMS, indicates that the K⁺ channels are more able to withstand various drastic treatments than the pump module or the excitation channels. Similar conclusions can be drawn from electrical measurements of Nitella protoplasts (1). While more exploration is needed, the SMS looks like a promising experimental system. It may be possible to improve the technique for the study of the pump state and excitation. The SMS are certainly very suitable for the K⁺ state.

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