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Conductometric Method for Detecting Ion Efflux during a Single Action Potential of *Chara*

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A simple device was developed to detect net ion efflux during a single action potential of *Chara* as an increase in the electric conductance of the bathing solution. The device showed a sufficiently high sensitivity and rapid response to the increase in the electric conductance. Net efflux of monovalent ions was estimated as 65–660 pmol cm^{-2} impluse^{-1} (average 220 pmol cm^{-2} impulse^{-1}).

Key words: Action potential — *Chara corallina* — Conductometry method — Ion efflux.

Internodal cells of fresh-water charophytes are well known for their ability to generate action potential. During the action potential, a transient increase occurs in the permeability for Cl^- (Findlay and Hope 1964). Large effluxes of K^+ and Cl^- were detected in internodes of various characean species (Gaffey and Mullins 1958, Mullins 1962, Hope and Findlay 1964, Haapanen and Skoglund 1967, Oda 1975). Oda (1976) simultaneously measured the amounts of K^+ and Cl^- flowing out of the *Chara corallina* cell during excitation, using flame photometry for K^+ and Ag-AgCl electrode method for Cl^-. He estimated both K^+ and Cl^- effluxes as 1,000–10,000 pmol cm^{-2} impulse^{-1}. Since the membrane capacitance is around 1 μF cm^{-2} and the amplitude of the action potential is 100–200 mV, the measured effluxes of K^+ and Cl^- were about 100 times larger than those carrying the capacitative current across the membrane. Kikuyama et al. (1984) also measured K^+ and Cl^- effluxes during excitation of intact and tonoplast-free Characeae cells. A large K^+ efflux was observed in both intact and tonoplast-free cells. On the other hand, no Cl^--efflux was observed in tonoplast-free cells.

To examine the ionic relations of cells during action potentials in Characeae, it is nescesary to measure K^+ and Cl^- efflux with a sufficiently high time-resolution. Haapanen and Skoglund (1967) developed a high-frequency reflectometry method to record an ion efflux during a single action potential of *Nitellopsis*. While highly complicated, their method was based upon the measurement of the electric conductance of the bathing solution. We developed a simple conductance meter that measures the electric conductance or its change in the solution with high accuracy. With it, we estimated the ion efflux during a single action potential of *Chara*.

Materials and Methods

Chara corallina cultured in the laboratory was used in the studies described here. Internodes isolated from neighboring cells were kept in APW containing 0.1 mM each of KCl, NaCl and

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Abbreviation: APW, artificial pond water.

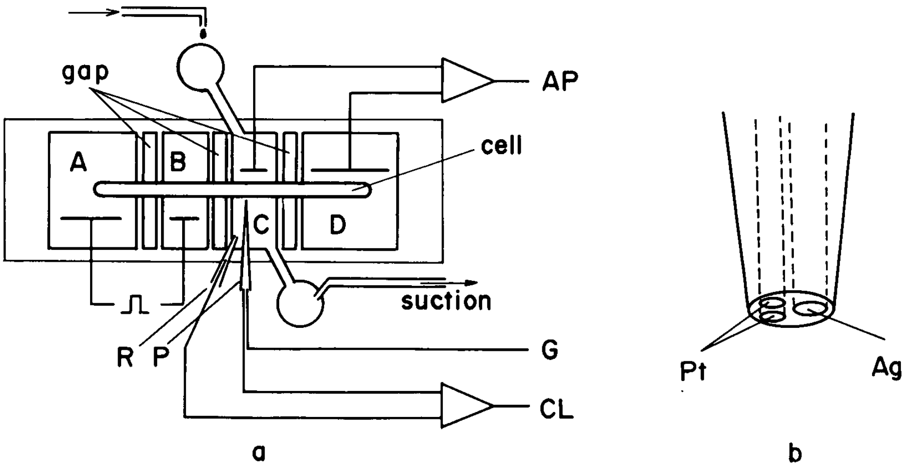

Fig. 1 **a** Setup of the measuring system. An internode was placed in a measuring vessel with four pools A, B, C and D which were separated from each other with air gaps. A stimulating electric current was applied between A and B. An action potential (AP) at C was monitored against D. A probe (P) was used to detect the electric conductance (G) and E_{Cl} (CL) which was measured against a reference electrode (R). Three pools A, B and D were filled with APW while C was continuously perfused with APW at a rate of 1 ml min^{-1}. **b** Enlarged illustration of the probe tip. The surface of Pt and Ag was covered with platinum black and AgCl, respectively. Tip diameter of the probe was about 1 mm.

$CaCl_2$. The cell was placed in a vessel with four pools, A, B, C and D, which were separated from each other by air gaps (Fig. 1). During the experiment each pool was filled with APW and pool C was perfused with APW at a rate of flow of about 1 ml min^{-1}. Changes in ionic concentrations following the induction of an action potential in pool C were measured.

A stimulating electric current was applied between pools A and B. If the amplitude of the pulse was sufficiently high, an action potential induced in B propagated to C and D. This method had the advantages of minimizing possible artifact in the measurements. Excitation at C was monitored against D with a pair of Ag-AgCl electrodes placed in both pools.

To measure Cl^- and total ion concentration, a special probe was designed (Fig. 1b). Three wires, a pair of Pt wires (0.1 mm in diameter) and an Ag wire (0.5 mm in diameter), were embedded in a polyacrylate rod (about 1 mm in diameter). The tip of the rod was ground with fine carborundum to expose the tips of the Pt and Ag wires which were then coated with platinum black and AgCl, respectively. The same Ag-AgCl wire was used to determine Cl^- concentration by the potential difference between Ag-AgCl and a reference electrode which was constructed with an agar-salt bridge connected to a calomel electrode via saturated KCl. The potential difference thus measured will, hereafter, be called the Cl^--potential (E_{Cl}). The pair of Pt-Pt black wires was used to measure the electric conductance of the bathing medium. The distance between the tip of the probe and the cell surface was about 0.1 mm.

A schematic diagram of the conductance meter is shown in Fig. 2. An AC voltage signal generated with a sine wave oscillator (OS) was applied to the probe (P). In this experiment, the frequency was fixed at 1 kHz and the amplitude was 15–35 mV peak-to-peak. The applied signal was monitored with a differential amplifier (A1; constructed with three operational amplifiers AD542). An electric current passing through the probe was monitored with an amplifier (A2; AD542) through 1 or 10 MΩ resistor. A 1 kHz band pass filter (BPF) was used to delete noise components in output of A2. The applied AC signal or electric current signal were converted into DC (root-mean-square value; rms value) with rms-to-DC converters (RMS1, RMS2; AD536A) and recorded with a pen-writing recorder. To detect small changes in the

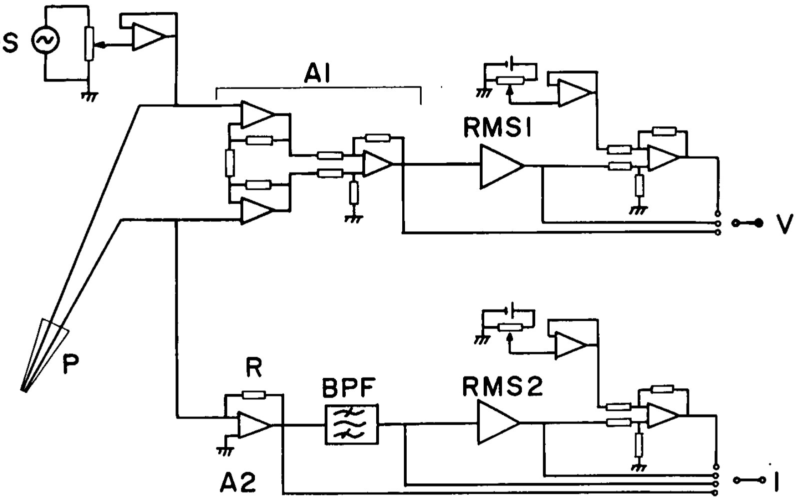

Fig. 2 Schematic diagram of the conductance meter. For details, see the text.

electric current, the output of RMS2 was measured under high amplification after subtracting the DC value from it.

Experiments were carried out at room temperature, 18–25°C.

Results and Discussion

The time resolution of the conductance meter was studied using a dummy circuit constructed with a phototransistor and photodiode. When an electric pulse was applied to the photodiode, the electric conductance of the phototransistor increased rapidly. The electric current (upper trace in Fig. 3) measured with A1 in Fig. 2 increased instantaneously with the application of the electric current pulse to the photodiode (lower trace in Fig. 3). Though the rms value (middle trace in Fig. 3) increased slowly, it reached a steady value within 10 ms. This indicates that the conductance meter can respond quickly to transient changes in electric conductance.

In APW, the phase of the AC current was not significantly different from that of the AC

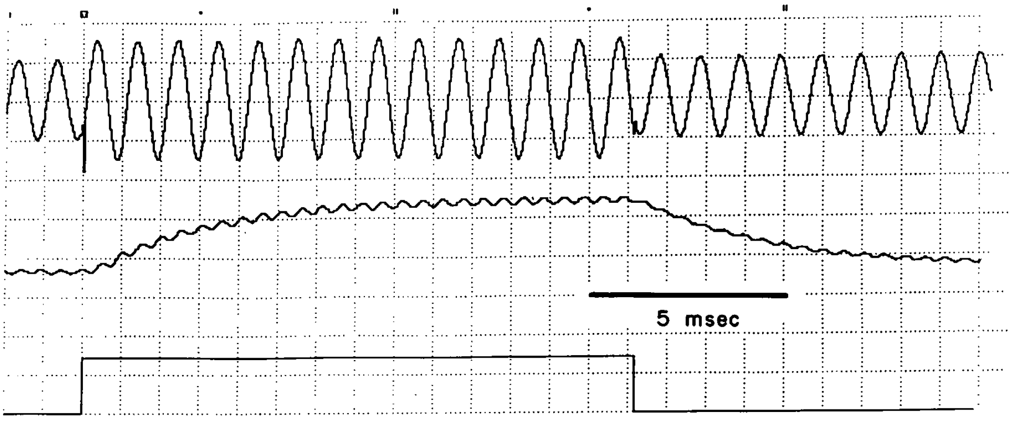

Fig. 3 Response of the conductance meter to a transient change in electric conductance in a circuit constructed with a phototransistor and a photodiode. When a DC current (lower trace) was applied to the photodiode, the electric conductance of the circuit increased instantaneously, as shown by the simultaneous increase in the amplitude of the current (upper trace). Though slower, the rms value (middle trace) reached a steady value within 10 ms.

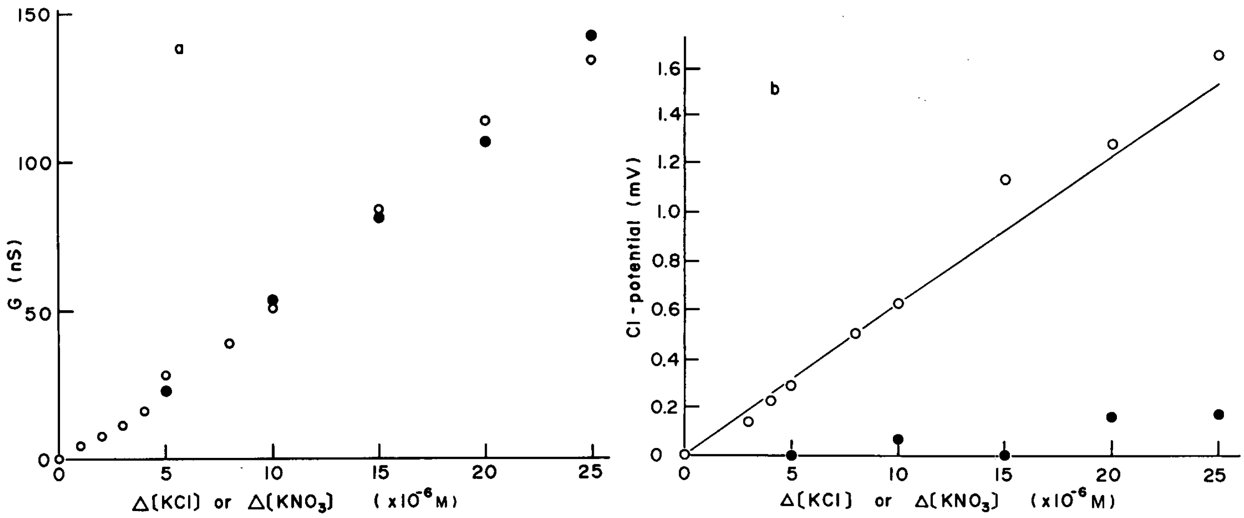

Fig. 4 Response of electric conductance (a) and E_{Cl} (b) to the addition of KCl (open circle) or KNO_3 (closed circle) to APW. In (b), a theoretical line calculated from Nernst's equation is also shown.

voltage applied to the probe. This indicates that the value of the electric current in APW reflects solely the electric conductance of the solution.

The electric conductance of APW apparently changed when the distance between the probe and the cell surface was altered, indicating that the topological arrangement of the probe and the cell should not vary during the experimental and calibrating processes. Calibration was carried out after each experiment by exchanging the perfusing APW to APW containing KCl in known concentrations. This was done to minimize the possible change in the topological situation of the probe and the cell. An example of such a calibration curve is shown in Fig. 4. For the increase in KCl or KNO_3, the increase in electric conductance was constant irrespective of ion species (Fig. 4a). On the other hand, E_{Cl} responded only to the increase in KCl (Fig. 4b).

Upon electrical stimulation, an internode generated a conducting action potential which was accompanied by a transient increase in electric conductance and E_{Cl} (Fig. 5). This is also seen in Table 1, cell 1. Although the local electric current compounded with the action potential to disturb the record, especially the record of E_{Cl}, it changed in a manner similar to that of the electric conductance (Fig. 5a). On the second excitation, an insignificant amount of Cl^- efflux was detected though the total ionic concentration increased as it did in the first excitation

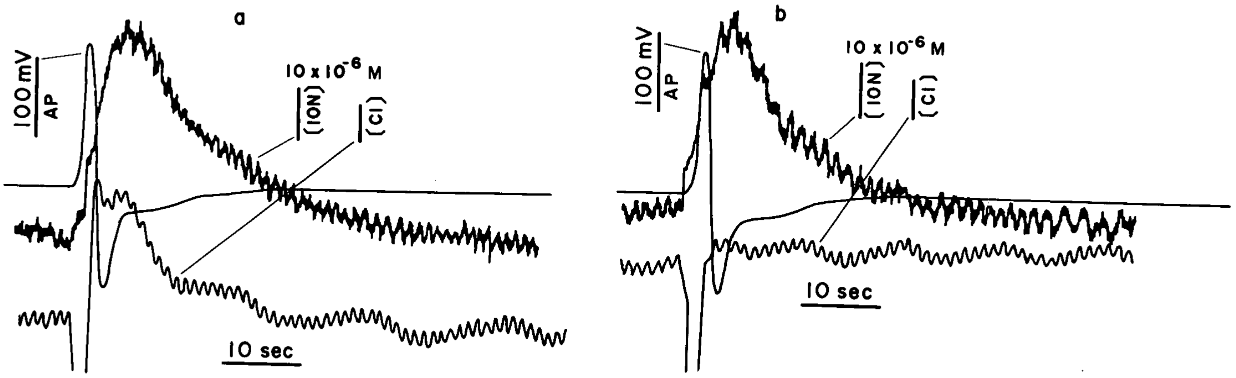

Fig. 5 Simultaneous record of the action potential (AP), change in the electric conductance ([ION]) and that of E_{Cl} ([Cl]). **a** When *Chara* cell generated an action potential, [ION] and [Cl] increased transiently. **b** On the second action potential, [Cl] changed insignificantly while the change in [ION] remained nearly constant.

Table 1 Effluxes of Cl^- and total ions during a single action potential of *Chara*

Cell No.	Action potential (mV)	Efflux (pmol cm^{-2} $impulse^{-1}$)	
		Cl^-	Total ions
1	150	450	660
	180	ND[a]	580
2	160	110	210
3	156	160	150
4	160	170	260
5	180	44	75
6	140	120	100
	190	110	90
7	183	67	87
	194	72	107
8	183	131	131
9[b]	150	105	280
10[b]	153	420	165
	174	130	200
average		149	220

[a] Not detected.
[b] Measured in standing APW.

(Fig. 5b). A similar phenomenon of an action potential with K^+ efflux but with no detectable Cl^- efflux was also reported by Kikuyama et al. (1984). They suggested that part of the difference between K^+ and Cl^- efflux may be accounted for by the influx of Ca^{2+} because it significantly increased during excitation of Characeae, as reported by other researchers (Hayama et al. 1979, Williamson and Ashley 1982, Kikuyama and Tazawa 1983). Since the electric conductance of the bathing medium was measured in this experiment, the difference between effluxes of total ions and Cl^- should be accounted for by effluxes of salts without Cl^- and/or by the increase in H^+ or OH^- efflux, the mobility of which is several times larger than that in other ions such as K^+, Na^+ and Cl^-.

Total efflux of ions (J) can be calculated with the following equation, assuming that ions are distributed equally in the space between the cell surface and the probe;

$$J=(R\times\int_0^\infty \Delta C\,dt)/A \qquad (1)$$

where ΔC is an increase in the ionic concentration, R is the rate of flow of the bathing medium through the space between the cell surface and the probe, and A is the area of cell surface facing the probe. Because it was almost impossible to determine R in our experiment, we used another equation to calculate the efflux by assuming that the medium between the cell surface and the probe is virtually static ($R=0$ in Eq. 1),

$$J=D\times\Delta C \qquad (2)$$

where ΔC means the maximum change in the ionic concentrations and D is the distance between the cell surface and the probe. Table 1 shows values calculated with Eq. 2. It also includes efflux data measured in standing APW as cells 9 and 10 in which Eq. 2 may strictly reflect ion effluxes. Since values of cells 1–8 are similar to those in cells 9–10, it can be assumed that the discrepancy between values calculated with Eq. 1 and Eq. 2 would be small.

Effluxes of Cl^- and total ions were in the range of ND (not detected) to 450 pmol cm^{-2} $impulse^{-1}$ (average 149 pmol cm^{-2} $impulse^{-1}$) and 65–660 pmol cm^{-2} $impulse^{-1}$ (average 220 pmol cm^{-2} $impulse^{-1}$), respectively, which were similar in order of magnitude to those reported previously (Kikuyama et al. 1984).

In Table 1 Cl^- efflux is sometimes larger than total ions extruded. This may be accounted for as follows. Changes in the electric conductance and E_{Cl} were measured after repetitive exchanges of the bathing medium from APW to APW plus 10 μM KCl and vice versa. During measurement, the tip of the probe was kept about 0.1 mm from the cell surface. After 16 exchanges of bathing medium, the change in the electric conductance was in the range of 25–41 nS (32±1 nS; mean±SD). On the other hand, that in E_{Cl} was in the range of 0.08–0.74 mV (0.43±0.04 mV; mean±SD) which corresponds to an increase in 1–12 μM (6.8±0.6 μM; mean±SD) in Cl^- according to the Nernst's equation. A large variation in E_{Cl} may reflect possible staining of the Ag-AgCl surface with substances on the cell surface because the electrode was very small and placed almost in contact with the cell. In these experiments, therefore, the average value of E_{Cl} may be more important than individual ones.

To study ionic behavior during excitation, flux measurements using isotopes such as ^{42}K, ^{36}Cl and others have been widely used (Browning and Nelson 1976, Findlay 1970, Gaffey and Mullins 1958, Mullins 1962, Hayama et al. 1979, Kikuyama et al. 1984). Flame-photometry and atomic abosrption spectroscopy have also been used (Oda 1976, Kikuyama et al. 1984). These methods have advantages in their high sensitivity and ability for detection and identification of ions. On the other hand, they are lacking or poor in the ability to analyze the kinetics of ionic behavior during an excitation. Ion selective electrodes are also to be powerful tools for studying ionic behaviors of cells because of their high selectivity and sensitivity to ions. Ag-AgCl electrode is widely used for this purpose since it responds very quickly to Cl^- (Mailman and Mullins 1966, Oda 1976, Kikuyama et al. 1984). For other ions such as K^+ and Na^+, however, there are no ion selective electrodes which respond faster than the excitation process.

Another method to detect the amount of ions is the measurement of the electric conductance of the solution (Small et al. 1975). Haapanen and Skoglund (1967) measured a transient increase in electric conductance during excitation of *Nitellopsis* with their high-frequency reflectometry apparatus. They estimated that KCl efflux was around 4,000 pmol cm^{-2} $impulse^{-1}$ by assuming that the increase in the electric conductance corresponded solely to the increase in KCl concentration. Our conductance meter also measured an increase in ionic concentration during an action potential by measuring the electric conductance of the solution. Since our device has a rapid response (Fig. 3) and high sensitivity (Fig. 4), it can be used to advantage in combination with other ion analysis methods for more detailed studies.

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