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Recording of Single K^+ Channels in the Membrane of Cytoplasmic Drop of *Chara australis*

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Summary

The cytoplasmic drop formed of effused cytoplasm from *Chara* internodes is enclosed by a membrane. Patch clamp experiments have been carried out on this membrane, revealing a K^+ channel as the most frequently detected ion translocator. The K^+ channel is saturated at a level of about 20 pA inward and 10 pA outward current. The channel conductance is dependent on the accessability of K^+ ions, its maximum value amounts to about 165 pS. The discrimination of Na^+ and Cl^- is significant, permeability ratios P_{Na}/P_K and P_{Cl}/P_K were estimated to be 0.01 either. Binding experiments with the fluorescent probe concanavalin A/FITC suggest that the membrane is derived from the tonoplast.

Keywords: Chara; Cytoplasmic drop; K^+ channel; Single channel; Tonoplast.

Abbreviations: E_K, K^+ equilibrium potential; FITC, fluorescein isothiocyanat; V_m, membrane voltage; V_{pip}, pipette clamp voltage; V_r, reversal voltage.

1. Introduction

The patch clamp technique (Neher 1982) is closely approaching the molecular mechanism of ion transport, and is demonstrated over a broad spectrum of different cell types and ionic channels in plasma membranes of animal cells (for survey see Kolb 1984). The difficulty to apply this technique to plant cell membranes is due to the existence of a cell wall surrounding the cytoplasm, thus preventing a tight sealing of the current measuring pipette with the membrane, which is indispensable for the observation of single channel current fluctuations. The problem may be avoided by forming protoplasts by enzymatic digestion of the cell wall. This preparation was accomplished by Moran *et al.* (1984) and Schroeder *et al.* (1984), being the first to present single channel events with patch clamp technique from the plasmalemma of leaf cell protoplasts. Another convenient model is the cytoplasmic drop isolated from Characean internodal cell, its membrane being of immediate access for the patch pipette. Kamiya and Kuroda (1957 a, b) published the method of preparation of such droplets, and demonstrated metabolic activity in the membrane-enclosed cytoplasmic compartment. Their method was modified by several groups to study the electrical behaviour of the membrane. Membrane voltage and resistance were shown to depend on the ionic composition and strength of the external medium (Inoue *et al.* 1973, Reeves *et al.* 1985). The membrane displays excitability (Inoue *et al.* 1973, Koppenhöfer and Schramm 1974). Illumination of the chloroplasts containing droplet causes changes in the cytoplasmic pH and membrane voltage, and inhibitor application indicates the existence of an active transport system to be resident in the droplet's membrane (Svintitskikh *et al.* 1985). Temperature significantly changes electrical and conformational state of the drop membrane, comparable to plasma membranes of intact cells, and Ca^{2+} concentration, as well, affects its function, obviously by inducing phase transitions of the lipid-protein complex between two different states of marked selectivity and high permeability to ions, respectively (Ueda *et al.*

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1974). So far, the cytoplasmic droplet is presenting a membrane with all interesting aspects of the intact cell. However, it is desirable to have the membrane characterized: is the boundary identical with the plasmalemma or the tonoplast? In literature one finds both interpretations for the membrane's origin. SVINTITSKIKH *et al.* (1985) consider the drop membrane as the original plasmalemma, REEVES *et al.* (1985) suppose that it is derived from the tonoplast. In recent communications, SAKANO and TAZAWA (1985, 1986) demonstrated that the membrane is of tonoplast origin. In this study, the patch clamp technique was used to attempt a characterization of K^+ channels in the membrane of the cytoplasmic droplet. Additionally, to reevaluate the observation of SAKANO and TAZAWA (1985, 1986), concanavalin A coupled with fluorescein isothiocyanat (FITC) was applied to the intact tonoplast by intracellular perfusion as a probe for drop membrane identification.

2. Material and Methods

Plant material. Chara australis R. Brown was cultured in plastic buckets under fluorescent tube illumination (Osram Fluora L 40 W/77) in a light/dark period of 14/10 hours at about 20 °C. For experiments, internodes were isolated and used without preincubation.

Cytoplasmic drop formation. An internodal cell was wiped dry with tissue paper and allowed to lose turgor by transpiration. Then, one end was cut and the opening submersed into a filtered (Millipore filter 0.22 µm) solution, which was approximately isotonic with the cell sap. In the bathing medium, drops formed from cytoplasm flowing out of the amputated cell. For patch clamp experiments, droplets with diameters of 150 µm or more were preferred. Sometimes, giant membranated lobed aggregations of cytoplasm could be observed at the bottom of the cuvette, containing rotating chloroplasts. If the bulk was not too large, it contracted within several minutes to a big droplet appearing spherical when top-viewed through the microscope, up to 600 µm diameter. There was no difference in channel activity before and after rounding off. The movement of particles or rotation of enclosed chloroplasts was taken as an indicator of metabolic activity in the cytoplasmic drop and the integrity of its membrane. After rupture of the droplet, the membrane deteriorated and chloroplast rotation ceased. Normally, the droplet survived some hours and could be patched repeatedly.

Solutions. The solutions prepared were of similar composition as those of KAMIYA and KURODA (1957 a). In the course of experiments, however, the feasibility of formation and the life-time of cytoplasmic drops turned out to be largely independent of the presence of single components, if only the isotonicity of about 300 mM ionic strength was provided. Instead of utilizing salts with NO_3^- as the anion (KAMIYA and KURODA 1957 a), chloride salts were applied. The basic solution contained (in mM): 77 KCl, 55 NaCl, 5.5 $CaCl_2$, and 5.5 $MgCl_2$. Na^+ and K^+ could be exchanged isoosmotically. To obtain a K^+ concentration greater than 150 mM without exceeding 300 mM ionic strength significantly, solutions were prepared from K_2SO_4. pH of either solution ranged at 5.9 ± 0.2, special buffer systems were not applied. For direct determination of the predominant channel in the excised patch mode, bathing medium and pipette solution contained different concentrations of KCl or combinations of KCl/NaCl. In cell-attached experiments, bathing solution and pipette filling were identical. All solutions, bathing medium and pipette filling were purified by microfiltering (Millipore 0.22 µm) before use. The experiments were carried out at 22 ± 2 °C.

Patch pipettes. In literature, much emphasis is put on the fabrication of patch pipettes, consisting in a 2-stage pulling and fire-polishing of the tip to obtain tight seals. Here, conventional glass capillaries (Hilgenberg, Malsfeld, F.R.G.) with an internally fused filament, providing quick-filling of the pipette, were used throughout all experiments. Furthermore, patch pipettes were pulled in a single step on a vertical pipette puller (David Kopf mod. 720, Tujunga U.S.A.),

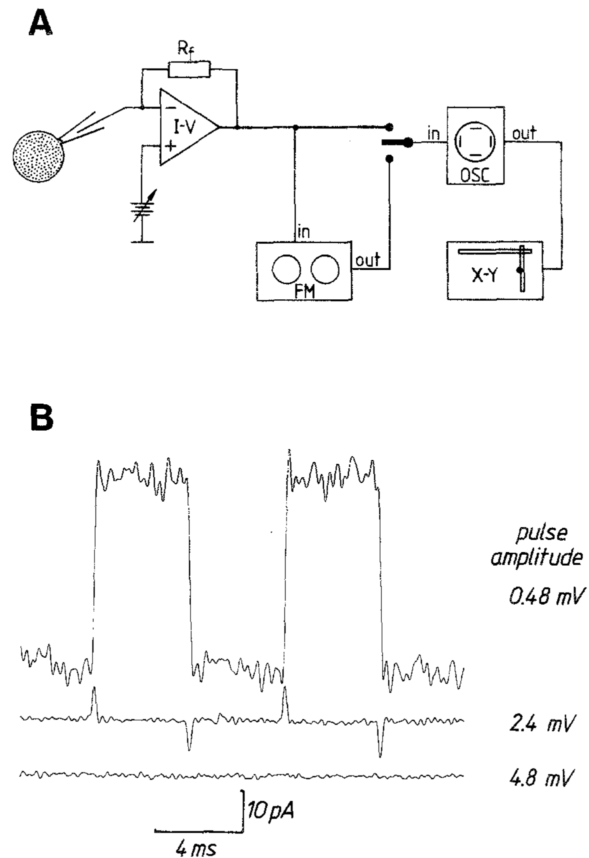


Fig. 1. *A* Scheme of experimental setup for single channel recording. After formation of a tight seal between pipette and cytoplasmic drop membrane, single channel currents are picked up and via a feedback resistor (R_f) converted into voltage signals by the patch clamp amplifier (*I-V*). The output was connected to a digital oscilloscope (*OSC*) serving as a monitor, and to a FM tape recorder (*FM*) in parallel. Recorded signals could be played back from the tape to the oscilloscope and the digitized data points be fed to a X-Y plotter (*X-Y*). *B* Recording of a typical sealing process. The upper trace shows current pulses fed through the open pipette (about 10 MΩ pipette resistance, the second trace shows the signal response after contact of the pipette with the droplet membrane and release of pressure on the pipette interior, third trace gives the signal response after compensation of capacitive spikes. Note the decrease in noise of the baseline

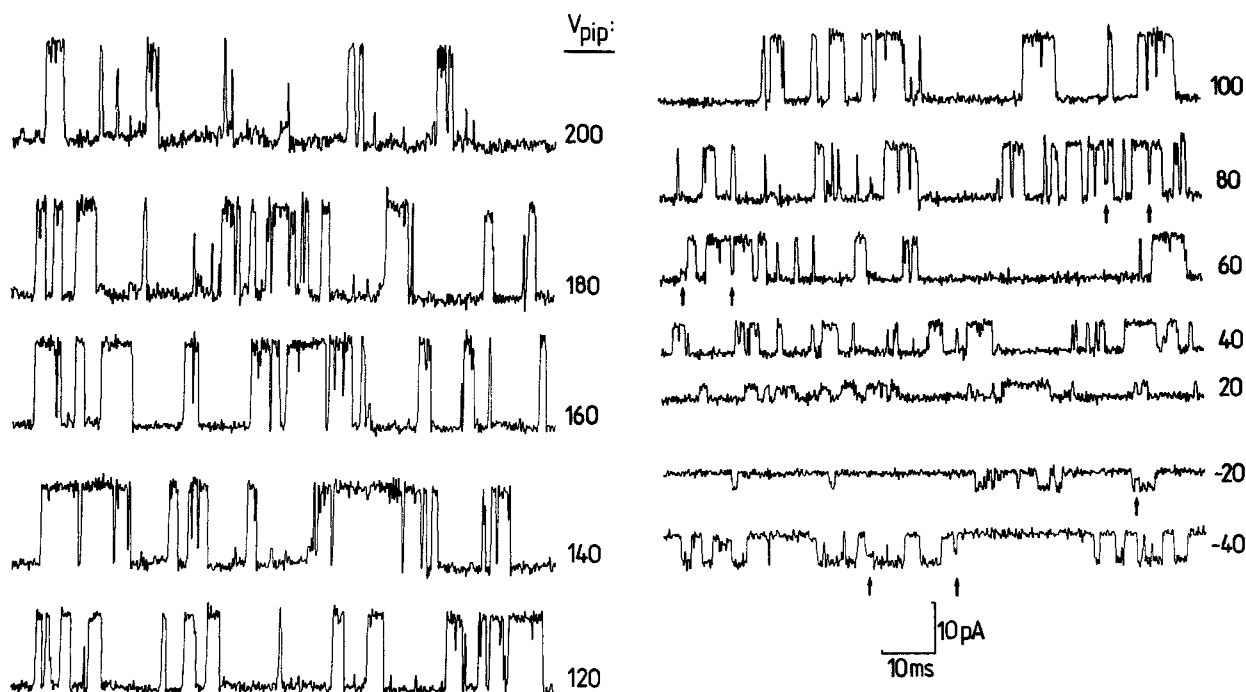


Fig. 2. Recording of single channel events from *Chara* droplets in the cell-attached mode. Clamped pipette potential (V_{pip} in mV) is indicated on the right-hand side of each trace. Note that at negative V_{pip} outward currents (downward deflections) and at positive V_{pip} inward currents (upward deflections) occur. Arrows indicate an intermediate opening channel (*subconductance level*). Bathing medium and pipette solution contained (in mM): 132 KCl, 5.5 $CaCl_2$, 5.5 $MgCl_2$

fire-polishing of the tip was omitted. The crucial point to obtain tight seals was the pipette diameter. Normally, seal resistance was in the range of 10 to 50 GΩ with pipettes having a tip resistance of about 10 to 20 MΩ. An example for a typical sealing process is given in Fig. 1 *B*. *Single channel recording*. After formation of cytoplasmic droplets in the measuring cuvette, a micropipette was placed at the membrane surface by means of a hydraulic micromanipulator, the driving head attached to a miniature manipulator (Narishige mods. MO-102 and MM-33, Tokyo, Japan). Sealing could be monitored on an oscilloscope (Nicolet mod. 3091, Offenbach, F.R.G.) by feeding rectangular current pulses through the pipette to ground. When a tight seal had formed by establishing a negative pressure at the pipette interior, *i.e.*, gently sucking, the current pulse disappeared and capacitive spikes were left (*cf.*, HAMILL *et al.* 1981). These capacitive currents could be abolished by the amplifier's internal compensation circuitry. The single channel recording device was commercially obtained (List mod. L/M-EPC-7, Darmstadt, F.R.G.). Measurement of channel activity took place in the cell-attached or excised patch mode; all signals were low-pass filtered at 3.3 kHz. For the different recording conditions see HAMILL *et al.* (1981). Briefly, inside-out patches were produced by drawing back the pipette from the droplet after formation of a tight seal, and exposing the resulting small vesicle at the tip to air for a short moment. The membrane portion facing the air will immediately disintegrate, whereas the membrane patch inside the pipette remains intact. Outside-out patches resulted from drawing back the pipette from the droplet, after the tight seal had formed and the patched membrane had been broken by a short sucking pulse. Thus, both sides of the membrane can be exposed to defined solutions. Signals due to channel activity were stored on a FM tape recorder with a frequency resolution greater than 3 kHz (Hewlett Packard mod. 3960, Frankfurt, F.R.G.).

To investigate the voltage dependence of single channel currents, the pipette was voltage-clamped. In case of cell-attached experiments, pipette clamp voltage (V_{pip}) is added to the original membrane voltage (V_m). Thus, at zero pipette clamp voltage channel behaviour at the original membrane voltage is observed. In Figs. 3 and 4, the voltage axis is displaying relative voltage ($V_m - V_{pip}$), whereas in excised patch mode (Fig. 5) the voltage axis displays the absolute voltage across the membrane patch, equalling $-V_{pip}$ in case of inside-out configuration, or $+V_{pip}$ in the outside-out configuration. After completion of voltage clamp series, data could be played back to the digital oscilloscope, and traces with opening and closing events were stored and hard-copied on a X-Y plotter (Rohde & Schwarz mod. ZSK 2, München, F.R.G.) (Fig. 1 *A*). Amplitudes of voltage-dependent single channel currents were then measured graphically. *Membrane characterization*. *Chara* internodes isolated from adjacent cells can be internally perfused with the above described basic solution, containing 1 mg/ml concanavalin A/FITC (*cf.*, SAKANO and TAZAWA 1985, 1986). The general procedure of intracellular perfusion was described in detail by TAZAWA *et al.* (1976). In brief, after the cell had lost turgor, both ends were cut and about 50 µl of the medium perfused from the slightly elevated opening to the lower one. When the solution had completely perfused the cell, it was ligated with polyester threads at both ends and the turgor restored by external hypotonic solution (200 mM sorbitol). About 15 minutes later, the cell was perfused again with basic solution but without concanavalin A/FITC and the free lectin washed off the vacuolar space. The secondly ligated cell contained now exclusively membrane-bound fluorescent probe, *i.e.*, the tonoplast being the only attainable membrane. In the fluorescence microscope (Zeiss Photomikroskop III, Oberkochen, F.R.G.), the cytoplasmic drop could be examined for fluorescence.

3. Results

Voltage-dependent channel currents and channel identification. The potential difference across the droplet membrane is given as the inside (cytoplasmic) potential compared to the external (pipette) potential. If not designated as pipette potential (V_{pip}, referred to ground, see Fig. 2), the voltage is noted as the negative clamped pipette potential ($-V_{pip}$) in case of cell-attached (Figs. 3 and 4) and excised patch measurements ($+V_{pip}$ in outside-out configuration) (Fig. 5). In the cell-attached mode, the negative applied clamp voltage is added to the voltage originating from the actual electrochemical gradients across the cytoplasmic drop membrane. Thus, at zero clamp voltage, channel behaviour at the original membrane voltage is reflected. Fig. 2 shows single traces of opening and closing events of the predominantly detected channel at different clamped pipette potentials. Although in several experiments single channels of markedly lower conductance could be observed, the channel described here was singular in amplitude and voltage dependence, and thus could unambigiously identified under different experimental conditions. Clearly, the amplitude of current fluctuation is voltage-dependent over a range from about + 150 mV to —70 mV (V_{pip}) deflection from original membrane voltage (V_{pip} = 0 mV). The experiments at either patch were highly reproducible, as demonstrated in Fig. 3 by four individual recordings. There were three parameters that could be evaluated, reversal voltage of the channel, its conductance, and saturation current through the channel. In experiments applying the cell-attached mode, pipette solution and bathing medium of the droplet were identical to prevent different electrochemical gradients across the patched membrane and the droplet surface residue, either, which might lead to some interference between the contiguous areas. In basic solution, inward current saturated at —100 mV, outward current saturated at about + 70 mV, superposing the resting voltage. With increasing extracellular K^+ concentration, the saturation phase of inward currents was shifted in negative direction (*cf.*, Fig. 4). The amplitude of these saturating currents is about 20 to 25 pA for inward current, that of outward current amounts to about 10 pA, measured in the cell-attached mode. With 77 mM KCl and in the presence of Na^+, saturation occurs at 9 pA and 5 pA of inward and outward current, respectively. Though the Na^+ concentration is decreased to the same extent as K^+ is increased, the saturation level rises (Fig. 4). In the presence of 77 mM K^+ in the bathing medium and pipette solution, the current-voltage curve displays a

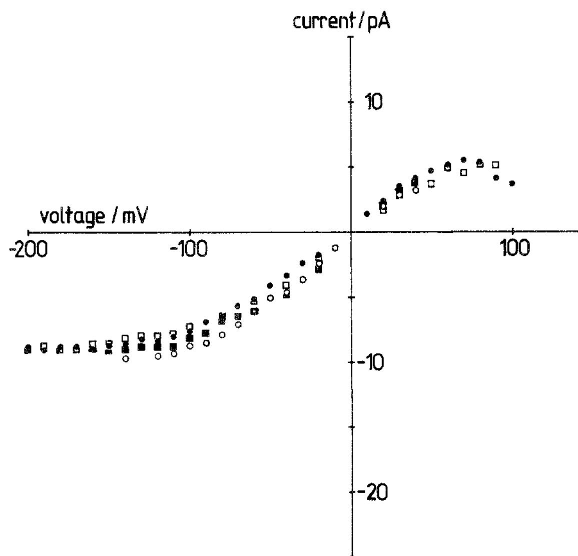


Fig. 3. Current-voltage relationship obtained from four different single channel recordings at different cytoplasmic droplets in the cell-attached mode. The voltage axis displays the negative pipette clamp voltage, the actual membrane voltage is 0 mV. Bathing medium and pipette solution contained (in mM): 77 KCl, 55 NaCl, 5.5 $CaCl_2$, 5.5 $MgCl_2$

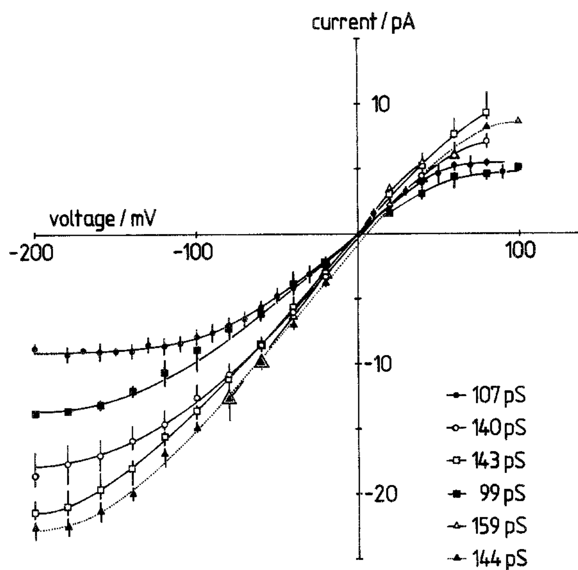


Fig. 4. Current-voltage relations of the putative K^+ channel at different K^+ concentrations in the bathing medium (= pipette solution). All data points are mean values (± S.D.) from at least four individual channels recorded in the cell-attached configuration under the respective condition. The voltage axis displays the negative pipette clamp voltage, regardless of the actual membrane voltage (membrane voltage determination at different conditions see discussion). Right-hand side, channel conductances at different K^+ concentrations at either reversal voltage are given. The different symbols represent the following K^+ concentrations in the bathing medium and pipette (in mM): ● 77 K^+ (+ 55 Na^+), ■ 77 K^+ (—Na^+), ○ 132 K^+, □ 150 K^+, △ 200 K^+, ▲ 185 K^+. All curves were recorded in the cell-attached mode

conductance of 107 pS and 99 pS, depending on whether Na^+ is present or not, whereas in solutions containing either 132 or 150 mM K^+, the conductance is about 140 pS. In a bathing medium containing 77 mM K^+ but no Na^+, current saturation was at a higher level as in the medium containing Na^+, the inward current increased from 9 to 13.5 pA. Fig. 6 shows that both, outward and inward current saturation, and single channel conductance measured in the cell-attached configuration are depending on K^+ concentration. Outward current saturation is difficult to obtain, since in most cases the membrane irreversibly breaks down during application of clamp voltages exceeding + 100 mV. Membrane patches were excised after assurance by a cell-attached measurement that the studied channel was present. The excised patch in inside-out or outside-out configuration shows a certain higher conductance in the range of 145 to 165 pS, the reason for this increase cannot be explained at this time. Furthermore, the isolated membrane patch is much more sensitive to high voltage clamp levels and often be destroyed at pipette potentials exceeding + 120 mV. The advantage of working with excised patches, however, is obvious, since both sides of the membrane are in contact with solutions of well-known composition. Therefore, the Goldman equation can be applied, and from measuring the reversal voltage (V_r) of the channel, the channel species can be directly determined, additionally, its selectivity (here α and β, respectively) to other ions may be calculated.

$$V_r = \frac{R \cdot T}{F} \ln \frac{[K^+]_o + \alpha \cdot [Na^+]_o + \beta \cdot [Cl^-]_i}{[K^+]_i + \alpha \cdot [Na^+]_i + \beta \cdot [Cl^-]_o}$$

with R, T, and F of their usual meaning, α and β as the ratio of permeabilities P_{Na}/P_K and P_{Cl}/P_K, respectively, and the subscripts *o* and *i* indicating outside and inside of the respective ion concentration. In this case, the above equation should be used for the actual conditions at the examined excised patches. The reversal voltages at different concentration gradients across the membrane were determined as —11 mV ($[KCl]_o/[KCl]_i$ = = 100/150), —17 mV ($[KCl]_o/[KCl]_i$ = 50/100) and ($[NaCl]_o/[NaCl]_i$ = 100/50), and + 19 mV ($[KCl]_o/[KCl]_i$ = 150/70), respectively (Fig. 5). According to the Goldman equation, the selectivities α and β could be calculated as 0.01 for either ion species, Na^+ and Cl^-.

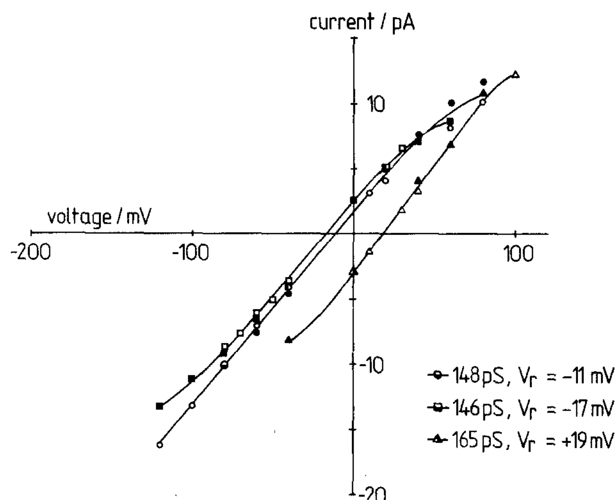


Fig. 5. Current-voltage characteristics of individual single channel recordings in excised patch mode. Data were obtained from two recordings (open and full symbols) at either conditions in inside-out or outside-out configuration, curves were fitted to the respective data points. Bathing medium/pipette solution contained (in mM): ⊖ 150 KCl/100 KCl (inside-out), ⊟ 50 KCl + 100 NaCl/ 100 KCl + 50 NaCl (outside-out), ▲ 150 KCl/70 KCl (outside-out). Channel conductance and reversal voltage at either condition are given at the right-hand side

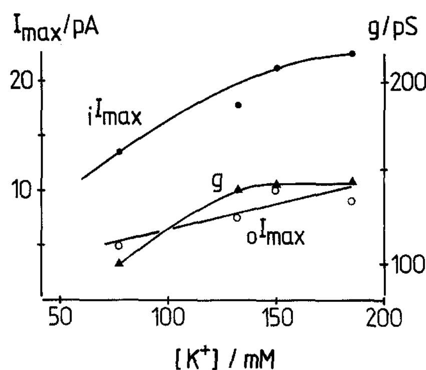


Fig. 6. Dependence of inward saturation current ($_iI_{max}$), outward saturation current ($_oI_{max}$), and conductance at reversal voltage (*g*) on external K^+ concentration. Data were obtained from cell-attached measurements, each point represents the mean value from at least four individual recordings

Different states of the channel. As indicated in Fig. 2 by arrows, there is at least one subconductance state of the investigated channel. The occurence of such states is described by several authors working on animal cell plasma membranes (for survey SACHS 1983). By chance it could be observed that the measured channel opened to a certain level and then reached a second one with significant higher conductance. Vice versa, a channel closing was only partial to a certain low conducting level, but still clearly above the baseline, then the channel returned to full aperture. It is easy to decide that no other channel of different species is involved, because the probability of exact coincidence of opening and closing of two different channels is extremely low.

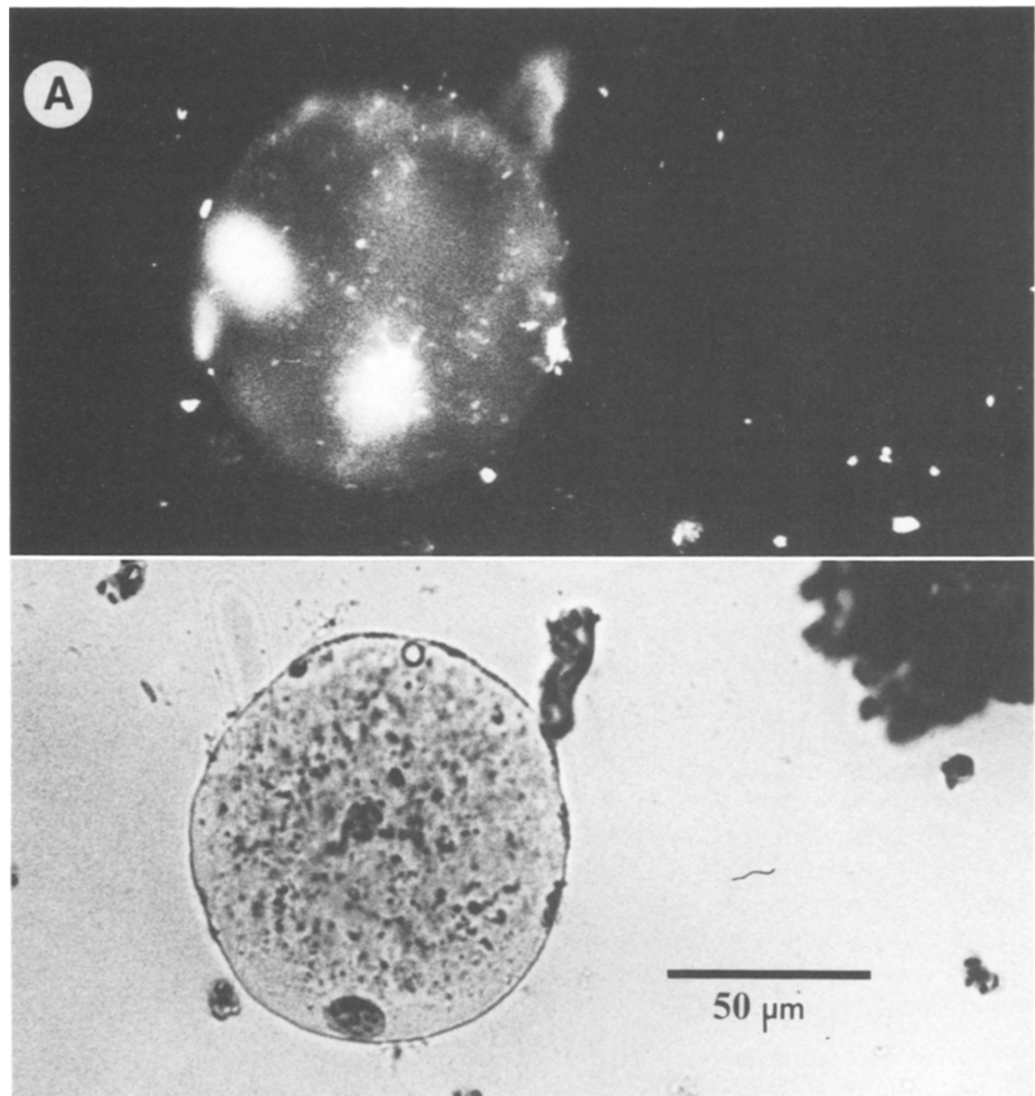
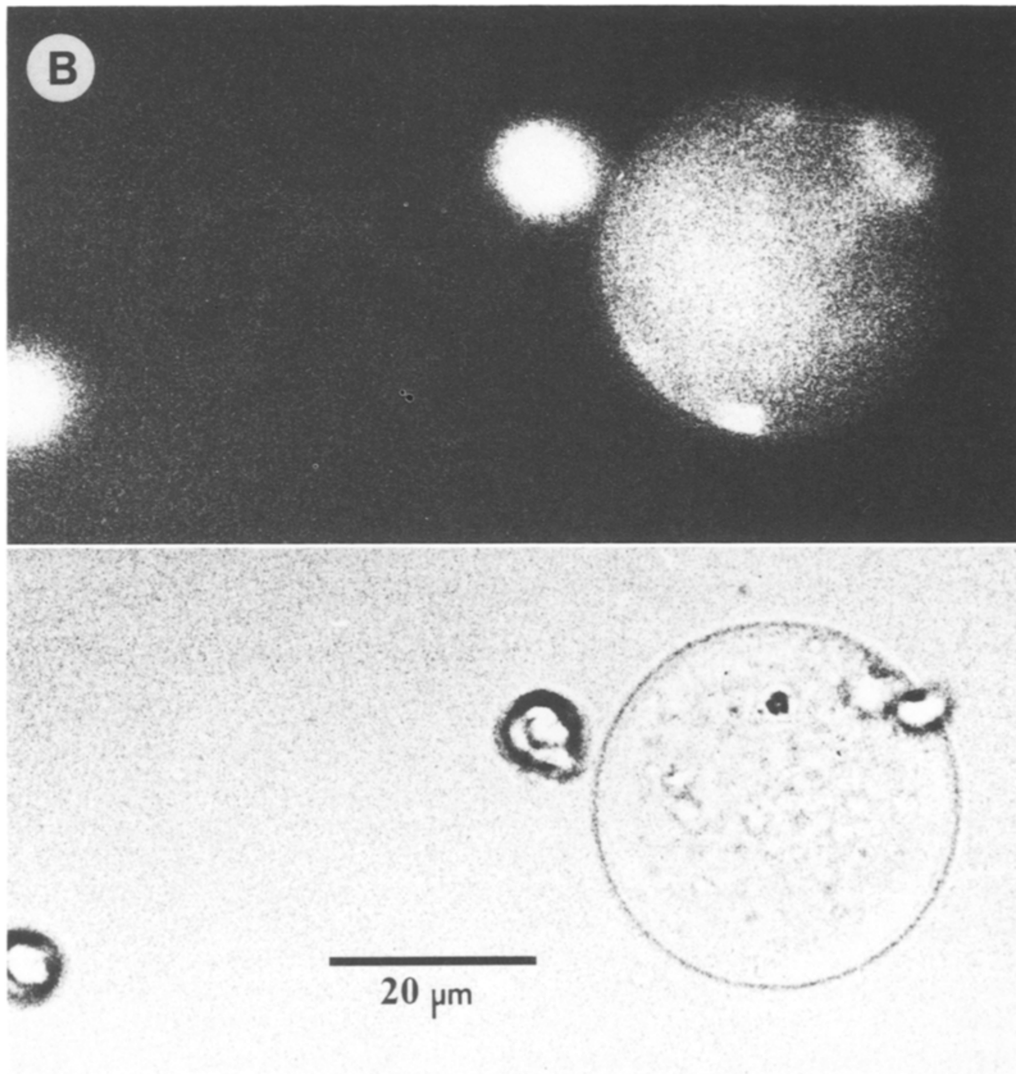


Fig. 7. Characterization of the cytoplasmic droplet membrane by fluorescent probe. Concanavalin A/FITC was formed after this treatment showed fluorescence. Lower photographs are the light micrographs corresponding to upper right corner). The locally enhanced fluorescence in *A* may derive from tonoplast fragments attached to the

Furthermore, this subconductance state seems to be voltage-dependent. Unfortunately, the limited amount of available data allows no valid statement at this time.

Membrane characterization. When a Characean internode is perfused, only the vacuolar sap is substituted for the applied artificial solution. The displaced original fluid is clear and without any particle in it. Sometimes, if perfusion medium rushes vigorously through the vacuolar space, it happens that the tonoplast breaks. Then, the effused cell content consisting of vacuolar sap and endoplasm appears as a milky fluid, usually containing visible particles. Here, *Chara* internodes were carefully perfused with the medium containing concanavalin A/FITC. Since the vacuolar face of the tonoplast alone was accessible for the fluorescein-coupled lectin, a fluorescing cytoplasmic droplet membrane would indicate its origin from the tonoplast. Fig. 7 shows photographs of cytoplasmic droplets with the membrane displaying fluorescence. Note that the accumulation of chloroplasts in the upper right corner of Fig. 7*A* does not display fluorescence. The non-uniform fluorescence of this droplet may derive from attached tonoplast fragments. The same phenomenon was found by SAKANO and TAZAWA (1986). Fig. 7*B* shows a smaller droplet with an approximately uniform staining.



bound to the tonoplast of intact *Chara* internodal cells by intracellular perfusion technique. Cytoplasmic drops
the respective fluorescence micrographs above. Note the absence of fluorescence at the chloroplast aggregation (*A*
cytoplasmic droplet surface. In *B* a fluorescing droplet is depicted which shows an approximately uniform staining

4. Discussion

There are two recent publications being closely related to the present study. BENTRUP *et al.* (1985) report patch clamp data obtained from whole-cell recordings on the tonoplast of isolated vacuoles, however, single channel events were not presented in this work. KRAWCZYK (1978) communicated membrane formation at the tip of a microelectrode inserted into the cytoplasm of another Characean cell, *Nitellopsis obtusa*. In spite of a probable contamination of the pipette tip by insertion, single channel events were detected after sealing of the tip in the cytoplasm. However, the conductance of the recorded channel is low (about 10 pS) compared to that described in the present work and not being identified. Moreover, the origin of the membrane formed at the tip is not clear, although the author discussed it as plasmalemma. In this work, activity of an identified single channel is demonstrated (*cf.*, Fig. 2) on the droplet membrane of *Chara*, the membrane was characterized as the tonoplast.

Channel identification. The present investigation revealed one predominant channel species. This channel displays a marked conductance compared to other observed channels. Its ion specificity is concluded from

experiments carried out in solutions of different composition in K^+, Na^+, and Cl^-. Absence of Na^+ in the external medium had no diminishing effect on the channel current, whereas with increasing K^+ both, the conductance and the current saturation level increased towards maximum values (Figs. 4 and 6). These findings suggest that the observed channel is a K^+ channel. Comparison of measured reversal voltages on excised patches with the calculated respective Nernst equilibrium potentials for K^+ confirm the assumption that the channel is a K^+ translocator. The reversal voltages in excised patch configuration yielded approximately the theoretical values of potassium equilibrium potentials (Fig. 5). According to the Goldman equation, the selectivity of the K^+ channel comparing Na^+ to K^+ and Cl^- to K^+ is estimated to be about 0.01, in either case.

Saturating currents and conductance of the K^+ channel. The apparent saturation of inward current in the presence of 77 mM K^+ is obviously not limited by the channel capacity, but is rather due to a dilute K^+ concentration. The current-voltage curves at higher K^+ concentrations support this interpretation (Fig. 4), and true saturation seems to approach a translocation rate of about 20 to 25 pA (Fig. 6), corresponding to approximately 10^8 K^+ ions per second maximum fluctuation. With increasing K^+ concentration, conductance of the channel rises towards a maximum value of about 150 pS. A displacement of 10 mV from K^+ equilibrium potential would create a single channel current of about 1.5 pA, corresponding to about $10^7\,K^+ \cdot s^{-1}$, taking the observed conductance of 150 pS at the reversal voltage into account.

The increase of outward saturation current depending on K^+ concentration in cell-attached experiments is not as pronounced as the inward current increase, which can be attributed to a maintenance of K^+ concentration in the cytoplasmic drop for the most part. However, the actually raised saturation level could be due to K^+ influx. From the fact that current-voltage curves obtained from cell-attached experiments in bathing solutions with different K^+ contents intersect the voltage axis at about 0 mV clamp voltage (Fig. 4), it may be assumed that the free running membrane voltage of the cytoplasmic drop is close to K^+ equilibrium potential, since otherwise the channel should display some activity at 0 mV clamp voltage. To prove this, membrane voltage was determined at different external K^+ concentrations in the following way. In the current clamp mode, *i.e.*, membrane current had been held at zero, the patch was broken by a sucking pulse. In the moment, the pipette interior joined the cytoplasmic phase, actual membrane voltage could be monitored on the oscilloscope. The measured voltage jumps amounted to + 22 mV at 150 mM K^+, + 15 mV at 132 mM K^+, and 0 mV at 77 mM K^+, cytoplasm compared to the external solution. From the Nernst equation, the cytoplasmic K^+ concentration is calculated to be approximately 64 mM, 71 mM, and 77 mM, respectively. The calculated values are in good agreement with the data obtained by ionspecific microelectrode measurements of K^+ concentration in the cytoplasmic droplet (68 mM), reported by REEVES *et al.* (1985). Thus, it is reasonable to assume the original membrane voltage to be identical with K^+ equilibrium potential at either condition, and consequently, the current-voltage relationship depends on $(V_m - E_K)$.

Comparing the conductance of the K^+ channel in the droplet membrane (150 pS) with the conductance of that found by SCHROEDER *et al.* (1984) in the guard cell plasmalemma (37 pS), the significant difference suggests that they belong to different types of K^+ channels. Consequently, it is questionable to assume the same channel density in the droplet membrane as in the guard cell plasmalemma. At present, no valid data of patch area measurements at the cytoplasmic drop are available. As a first approach to estimate the patch area in the cell-attached configuration, the data presented by SAKMANN and NEHER (1983) are used. They consider the Ω-shaped membrane portion in the pipette to be in the range of 2 to 25 μm^2. Generally, the resistance of all pipettes I employed ranged at 10 MΩ. In the course of some experiments, several K^+ channels could be observed in the respective patch with their open-times overlapping and therefore current amplitudes adding up. From observations of the highest current level at high clamp voltage (V_{pip} + 100 mV to + 120 mV), the channel density was roughly estimated to be in the range of 2 to 4 μm^2/channel, considering the patch area to be of the order of 20 μm^2. Thus, the density is also clearly divergent from that found by SCHROEDER *et al.* (1984). It remains questionable whether the membrane which incorporates this putative new type of K^+ channel can be classified. The high conductance K^+ channel could be characteristic for the tonoplast, or secondly, it could be specific for Characean membranes, eventually, it could be characteristic for excitable membranes.

Comparison with in vivo investigations. Assuming a homogeneous K^+ channel distribution over the whole membrane area, and a density of 1 channel/4 μm^2, the flux of K^+ ions per unit area can be calculated from the

observed mean conductance of 150 pS. A displacement of 10 mV from the K^+ equilibrium potential would create a flux of 350 to 400 pmoles · $cm^{-2} \cdot s^{-1}$. This would correspond to the order of magnitude reported by RAVEN (1976) for K^+ fluxes across the tonoplast of *Chara australis*. The comparison requires similar electrochemical gradients across the tonoplast in *in vivo* and across the cytoplasmic drop membrane. This requirement is met by the composition of the artificial solutions, and secondly, by the natural potential difference across the Characean tonoplast of about —10 mV to —30 mV, the cytoplasm being negative compared to the vacuolar compartment, *i.e.*, near the K^+ equilibrium potential (RAVEN 1976). This is in good agreement with the state of the droplet's membrane in this study, where the resting voltage obviously is close to the K^+ equilibrium potential and the chord conductance determines K^+ flux (*cf.*, Fig. 4).

It was shown that membrane voltage and resistance of the plasmalemma (OKAZAKI *et al.* 1984) and excitability (ZIMMERMANN and STEUDLE 1978) are strictly depending on turgor. Since turgor develops across the plasmalemma and not the tonoplast, the present cytoplasmic droplet system is probably displaying an almost natural behaviour, not dealing with the problems inhering protoplast investigations.

Hitherto, with the present equipment it was impossible to evaluate mean open-times or even kinetics of single channel events. However, from oscilloscope observations it seems that the frequency of transitions between open and shut states is voltage-dependent. Furthermore, there are periods of inactivity, considerably longer than closed time during periods of open-close transitions. Therefore, it may be assumed that channel transitions occur in a burst-like manner (*cf.*, KRAWCZYK 1978). Analysis of channel kinetics is subject of a further investigation.

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