Plant Physiol. (1994) 106: 313–319

Depolarization-Activated K^+ Channel in *Chara* Droplets

Igor I. Pottosin[1] and Pavle R. Andjus[2]*

Institute of General and Physical Chemistry, P.O. Box 551, 11000 Belgrade, Yugoslavia

A novel potassium channel was characterized in the droplet membrane of *Chara gymnophylla*. This channel has a conductance of about 90 pS (in symmetrical 0.15 M KCl), which is lower compared to the 170-pS K^+ channel predominant in this preparation. In contrast to the large conductance K^+ channel, the novel channel opened with a delay at depolarization and closed at hyperpolarization and did not require cytosolic Ca^{2+} for its opening. It also showed comparatively weak selectivity for K^+ over other monovalent cations, although its cation to anion selectivity was high. Externally or internally applied Cs^+ blocked the channel in a voltage-dependent manner, similarly to the 170-pS channel. The sensitivity of the 90-pS channel to external tetraethylammonium chloride (half-blocking concentration approximately 1.5 mM) was 20-fold higher compared to the large conductance channel. With respect to its voltage-gating kinetics, the 90-pS channel was identified as a "slow delayed rectifier."

The membrane of the cytoplasmic droplet is easily patch-clamped, thus providing an ideal model system to study ion channels. Most common in this membrane is the large conductance (about 170 pS in 0.15 M KCl), K^+-selective channel, which could be activated by hyperpolarization (cytoplasmic side made negative) and micromolar cytosolic Ca^{2+} (Lühring, 1986; Laver and Walker, 1987, 1991; Bertl, 1989; Pottosin, 1990, 1992; Pottosin et al., 1993). An anion-selective channel of lower conductance was also described in this preparation (Tyerman and Findlay, 1989). However, there is increasing evidence for the presence of another K^+ channel type in the *Chara gymnophylla* droplet membrane, with lower conductance and different gating (Homble et al., 1987; Tyerman and Findlay, 1989). This channel closed at membrane hyperpolarization where the concomitant activation of the other K^+ channel type was observed. The K^+-selective nature of this channel was deduced mainly from its current reversal potential. In cell-attached patches a rare occurrence of this channel was reported (Tyerman and Findlay, 1989), whereas in the whole-droplet mode the macroscopic current produced by a population of such hyperpolarization-deactivated channels was prominent (Homble, 1987). No systematic study has been carried out yet to elaborate the experimental protocol that could allow for the separation of the current through these channels from the current passing through the most abundant K^+ channel type (170 pS).

In this patch-clamp study of *C. gymnophylla* droplets we were able to describe a K^+ channel type with gating controlled solely by membrane depolarization. This allowed us to examine the properties of the novel potassium channel type selectively by decreasing the free Ca^{2+} concentration at the cytoplasmic face of the membrane to the nanomolar range where the large conductance Ca^{2+}-activated channels were generally inactive (at least at membrane potentials greater than −100 mV).

MATERIALS AND METHODS

The green charophyte alga *Chara gymnophylla* was grown on sand in aquaria filled with dechlorinated tap water. The water temperature was regulated in the range of 20 to 25°C. The aquaria were illuminated for 12 h/d using fluorescent lamps (2700 lux). Although the algae were screened by wooden panels from natural light, some influence of ambient light was possible.

The internodal cells (10–30 mm in length, second to fifth from the plant top) were the source of droplets. The preparation procedure was described by Bertl (1989) with modifications (Pottosin, 1992). Briefly, both cell ends were cut, and the cell interior was perfused with a solution containing (in mM): 150 KCl, 10 Hepes-KOH (pH 7.2), 0.05 or 1 $CaCl_2$. This procedure led to immediate formation of round vesicles (30–60 μm diameters) containing cytoplasm. An aliquot of 100 mL of the preparation medium containing droplets was transferred to the experimental chamber on a Petri dish containing 3 to 5 mL of the same solution.

The microelectrodes were drawn into the experimental chamber under positive pressure; releasing the pressure near the droplet membrane caused the movement of the vesicle toward the pipette tip and eventually resulted in a tight seal (10–30 GΩ). The standard technique (Hamill et al., 1981) was further used to obtain inside-out and outside-out membrane patches. Microelectrodes were pulled (model 51217 pipette puller; Stoelting, Wood Dale, IL) from soft "molybdenum" glass microtubes or from hard borosilicate glass (GC150F-15; Clark Biomedical Instruments, Pangbourne, UK), and after fire polishing, their resistance was 4 to 6 or 7 to 20 MΩ, respectively. The electrode-filling solution was the same as in the bath (see above), but free Ca^{2+} was buffered to 1.5 nM using 1 mM EGTA and 5 mM K-citrate. Prior to an experiment the pipette solution was filtered with a 0.22-μm filter (Milli-

[1] Present address: Institute of Cell Biophysics, Pushchino, Moscow region, 142292, Russia.

[2] Present address: Scuola Internazionale Superiore di Studi Avanzati, Via Beirut 2–4, 34013 Trieste, Italy.

* Corresponding author; fax 39–40–3787528.

Abbreviations: DR, delayed rectifier; I/V, current-voltage; $K(0)$, zero voltage affinity; $K_{½}$, half-blocking concentration; P_x, permeability for ion x; SDR, slow delayed rectifier; TEA, tetraethylammonium chloride; V_m, membrane potential difference; z', effective valence.

pore, Bedford, MA). Soft glass low-resistance electrodes were used to obtain inside-out patches by electrode withdrawal in a low Ca^{2+} (0.05 mM) medium, and outside-out patches were obtained by breaking the membrane patch in the cell-attached mode and by pipette withdrawal. The resting droplet potential was also measured by current clamp in whole-droplet configuration. High-resistance borosilicate glass electrodes were used to obtain stable outside-out patches after the vesicle formed at the electrode tip was disrupted by applying a large negative potential (−200 mV for 1–2 s, pipette interior relative to bath). These outside-out patches usually contained a few or even one channel of interest, and the low capacitance of the thick wall electrodes (2–3 pF) enabled low-noise single-channel recordings. The selectivity measurements were performed with the excised patches in bi-ionic conditions where KCl in the bath was replaced by NaCl, RbCl, or CsCl. Channel block was analyzed by substitution of the bath solution with solutions supplemented with 10 to 15 mM CsCl or 1 to 5 mM TEA.

Recordings (filtered at 1 kHz, 4-pole Bessel) were made with the EPC-9 patch-clamp amplifier (Heka Elektronik GmbH, Lambrecht, Germany). Experimental control (monitoring, stimulation, acquisition, data storage) was performed with the "EPC-9 SCREEN" program (Heka) on an Atari Mega ST 4 computer. In pulsed experiments the command voltage (pipette relative to the bath) was provided in the form of rectangular or ramp wave pulses using the programmed pulse generator. Data were analyzed with the "Review" and "TAC" software packages (Instrutech, Elmont, NY). V_m was taken with respect to the outside of the membrane (cytoplasm minus exterior). The positive current is defined as the positive charge flowing from the inside of the membrane (cytoplasmic side).

RESULTS

General Observations

Two types of K^+ channels were identified in the membrane patches isolated from *C. gymnophylla* droplets. The first one was identified as the previously described Ca^{2+}-activated K^+ channel (Pottosin, 1990, 1992; Pottosin et al., 1993) with a conductance of 170 pS (±0.6%) in symmetrical 150 mM KCl. The other channel had a smaller conductance of 86 ± 12 pS (n = 14) in the same solution. The distinctive feature of the latter channel was its strongly voltage-dependent kinetics. In all of the patches examined (n = 42) it closed within several seconds when the potential was stepped below −30 mV and opened with a variable delay at potentials above +30 mV. Between −30 and +30 mV much slower activation-deactivation kinetics were observed, and once the channel opened it showed only infrequent and brief closures. This behavior was also preserved at larger negative potentials unless the prolonged closure occurred, whereas at V_m > +30 mV the channels showed flickering, which sharply increased with potential increase (Fig. 1, continuous recording). The large conductance channel, however, showed bursting behavior at positive and negative potentials as well (Fig. 1, ramp and continuous records). According to its gating behavior, the lower conductance channel was identified as a SDR.

The maximal expression of SDR was observed from March until the beginning of April. During this period the activity of large conductance Ca^{2+}-activated K^+ channels was greatly diminished, and the activity of SDR could be monitored without interference even at relatively high cytosolic Ca^{2+} (0.05 mM). However, beyond this seasonal period the patches usually contained about 10 Ca^{2+}-activated K^+ channels and only 1 to 3 SDR channels (not shown). Resting potential of droplets measured in this period was +5.8 ± 0.8 mV (n = 8), and by the beginning of April a bimodal distribution of V_m was observed between the above positive value and a negative value, finally leaving only the negative V_m of −24 ± 21 mV (n = 87). This seasonal behavior has been observed during the course of 2 successive years.

Generally, to separate the two channels we decreased the cytosolic Ca^{2+} concentration to the nanomolar range. Thus, the activation of the large conductance channel was shifted to large negative potentials (<−100 mV) (Pottosin et al., 1993). Figure 1 presents a rare case in which the activities of both channels were recorded simultaneously at low Ca^{2+} (the membrane patch contained at least three large conductance channels, but only a few single, short openings were recorded at this potential). As Figure 1 indicates, SDR did not require cytosolic Ca^{2+} for its opening. Furthermore, an increase of cytosolic Ca^{2+} concentration of up to 1 mM did not produce a significant effect on SDR kinetics (not shown). Also, the channel was not affected by 5 mM ATP added to the cytoplasmic side (inside-out patches, n = 3). Therefore, it is likely that the activity of SDR in isolated patches was controlled solely by membrane potential.

Rectification in the SDR Channel

Figure 2 shows the current response of the patch containing at least three channels to a voltage step from −60 to 60 mV and vice versa. Upon the depolarization step, the activation of SDR, initially silent at −60 mV, occurred with a delay; hence, the time course of activation in the averaged trace in Figure 2 (bottom) is sigmoidal. In contrast, immediate deactivation of the channels occurred upon a hyperpolarizing step, with a time constant of 2.45 ± 0.25 s (outside-out patches, n = 7). Therefore, it can be expected that the macroscopic current originating from these channels will show an outward rectification. However, the increase of closures at large positive potentials should tend to moderate this rectification. In addition, the I/V relationship of the open channel in drop-attached patches showed significant inward rectification, which was completely abolished after patch excision (Fig. 3). These I/V characteristics were similar to those obtained in the presence of a blocker (Cs^+; see below), which could imply that some cation present in the cytoplasm exerted the inhibitory effect. Figure 3, c and d, also shows that the increase of current upon patch excision was followed by a decrease of noise in the open state, which is exactly what could be expected for the relief of blockade.

Selectivity and Blockade of the SDR Channel

Selectivity was determined by measurements of the I/V relationship of the channel open state in outside-out patches

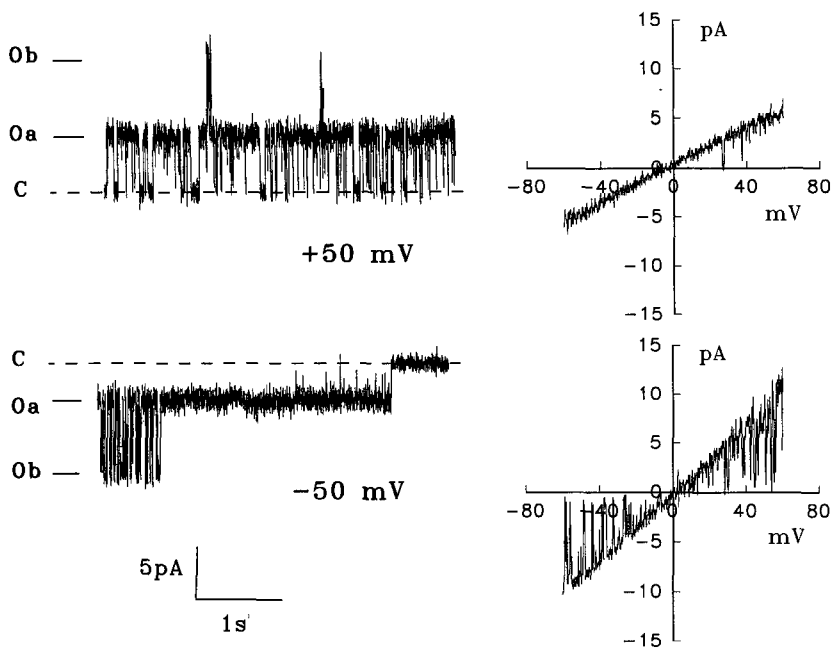

Figure 1. Examples of recordings of two types of channels on outside-out patches from the droplet membrane of C. *gymnophylla*. Right, I/V plots obtained from ramp-pulsed recordings (−60 to +60 mV, 500 ms) of the lower conductance (90 pS) channel, SDR (above), and of the higher conductance (170 pS) channel (Ca^{2+}-dependent K^+ channel). Left, Continuous recordings at membrane potentials (V) indicated below each trace. Openings of two channels differing in current amplitudes can be seen (Oa and Ob; the closed state is indicated by the horizontal interrupted lines). Ca in the bath solution was 1 mM, buffered to 1.5 nM in the pipette; symmetric 150 mM KCl.

under bi-ionic conditions (KCl in the bath was replaced by the same concentration of NaCl, RbCl, or CsCl). An example of such I/V curves is presented in Figure 4. Cation substitution at one membrane side caused a strong rectification of the current flow, indicating low conductivity of the present channel to alkali cations other than K^+. Measurements of the slope conductance of the inward current, which was produced mainly by cation influx from the bath, served as an estimate for channel conductivity to different cations. Thus, we estimated the mean conductance for Na^+ and Rb^+ of 9.0 pS (n = 8) and 4.7 pS (n = 17), respectively (n is the number of I/V traces used), whereas the conductivity to Cs^+ was found to be immeasurably low. However, the relative permeabilities of the above ions as determined by reversal potential measurements were significant, indicating a moderate selectivity of the channel for K^+ over monovalent cations. The following ratios of relative permeabilities were calculated using the Goldmann-Hodgkin-Katz equation (value of reversal potential ± SE in parentheses): $P_{Na}/P_K = 0.4$ (−23 ± 4 mV) and $P_{Rb}/P_K = 0.7$ (−8 ± 2 mV). The cation to anion selectivity of this channel was high, since the reversal potential of −30 mV measured in a transmembrane gradient of 150/50 mM KCl was close to the Nernst potential for K^+ (equilibrium potential for K = −28 mV). The relative permeability to Cs^+ could not be determined because there was no detectable current carried by this ion.

The inability to measure the Cs^+ current led us to investigate the possible inhibitory effect of this ion. Figure 5 shows typical I/V curves of the open channel obtained in the absence and presence of 10 mM Cs^+ at the external membrane side. There are three regions in the I/V curve with Cs^+: (a) between +60 and 0 mV, where the channel conductance was not affected; (b) between 0 and −90 mV, where strong current suppression and a region of negative slope conductance was observed; and (c) below −90 mV, where an upturn of current was observed, implying that Cs^+ was forced to pass through the channel, thus allowing the influx of K^+ ions. The middle part of this trace has been fitted to the Woodhull model of block (Woodhull, 1973) and gave the $K(0)$ for Cs^+ of 80 mM and the $z' = 1.35$. The latter value was larger than unity, which implied that at least two binding sites within the channel pore could be simultaneously occupied by external Cs^+ ions. A similar experiment on an inside-out patch revealed a weaker voltage dependence for the internal Cs^+ blockade, with $K(0)$ = 60 mM and $z' = 0.5$ (not shown).

We also examined the inhibitory effect of externally applied TEA on the open state current (Fig. 6). Unlike Cs^+, the blockade by TEA (1.5 mM) was voltage independent, and $K_{½}$ was 1.7 ± 0.4 mM (outside-out patches, n = 3).

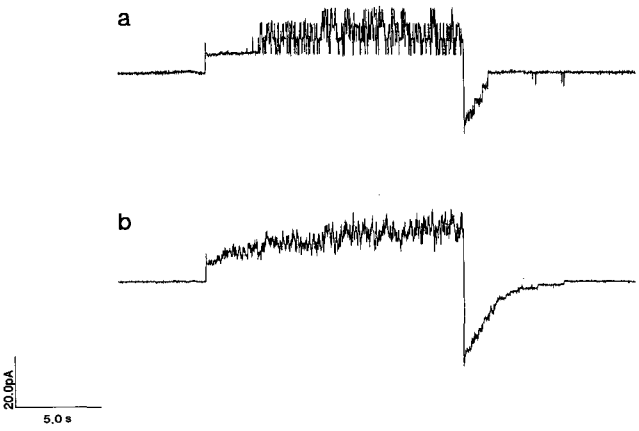

Figure 2. Current response of an outside-out patch containing at least three channels to the voltage step from −60 to 60 mV and back. a, Single-pulsed recording; b, averaged recording from four pulses. Ionic composition of solutions was the same as in Figure 1.

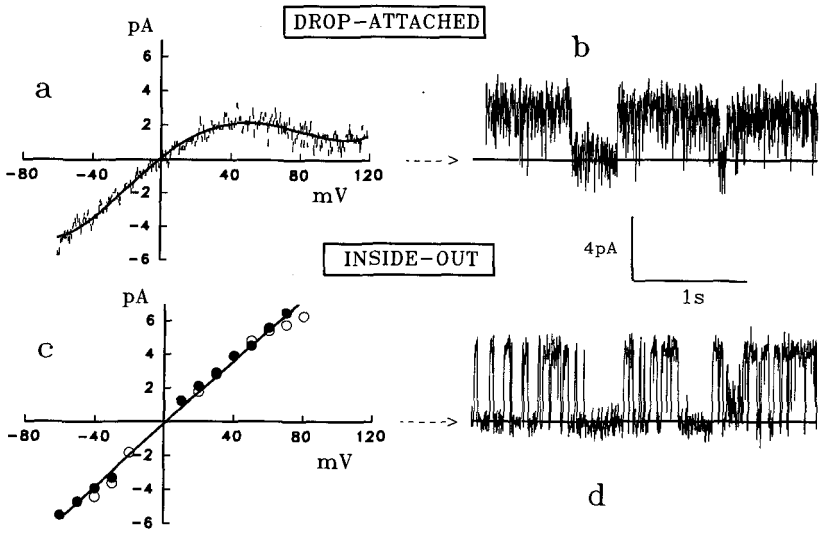

Figure 3. Inward rectification of the current through the single SDR channel in drop-attached patches and relief of rectification after patch excision. a, I/V plot obtained by a ramp-pulsed recording (−60 to +120 mV, 500 ms; interrupted line) on a drop-attached patch; continuous line is a polynomial fit to the ramp data; b, example of a continuous single-channel recording from a different drop-attached patch at V_m = 50 mV; the closed level line is aligned with the abscissa in a, and the current amplitude scaling is the same as for the ordinate in a; c, I/V plot of the channel open state for two different inside-out patches (i.e. open and filled circles; the line is a linear fit to all of the data); d, example of a continuous single-channel recording at V_m = 50 mV from the inside-out patch obtained upon excision of the drop-attached patch in b; the closed level line and the current amplitude scaling are adjusted according to the plot in c, analogous to b versus a. Ionic conditions were the same as in Figure 1, except in c and d, where Ca^{2+} in the bath was 0.05 mM.

DISCUSSION

Channel Identification

This study provides several lines of evidence for the presence of a second K^+ channel type in the *Chara* droplet membrane with specific properties distinguishing it from the previously described large conductance Ca^{2+}-activated K^+ channel (Pottosin, 1990, 1992; Pottosin et al., 1993). The novel K^+ channel described here (SDR) had approximately 2-fold lower conductance and showed a weaker selectivity. SDR opened at positive potentials where the large conductance channel was mainly closed and did not require cytosolic Ca^{2+} for activation. However, sensitivity to Ca^{2+} cannot be excluded since it was not checked in situ. Using intradroplet injection of Ca^{2+} while in drop-attached mode Katsuhara et al. (1991) increased the open probability of a K^+ channel resembling SDR in *Nitellopsis obtusa* (see below).

SDR also showed substantially higher sensitivity to exter-

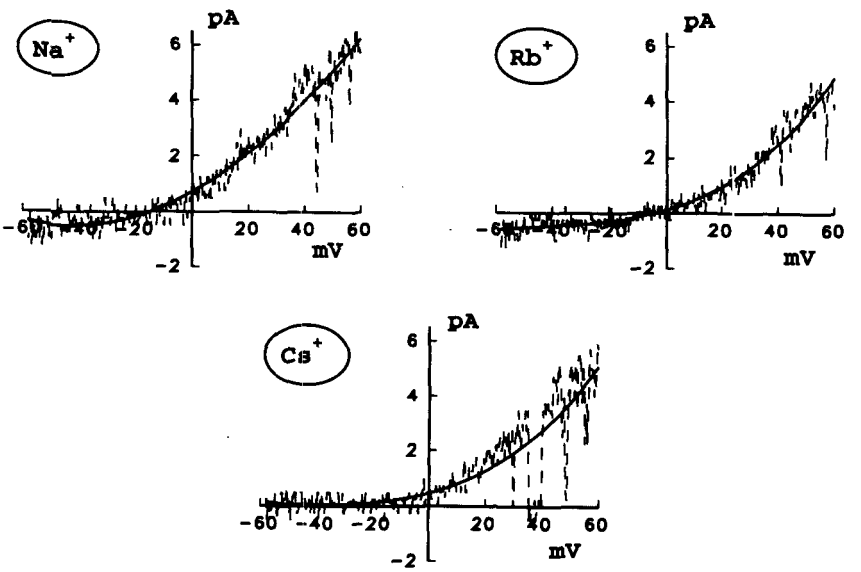

Figure 4. SDR channel selectivity: I/V plots from ramp-pulsed recordings (interrupted lines) on different outside-out patches in bi-ionic solutions where 150 mM KCl in the bath was replaced by the same concentration of NaCl, RbCl, and CsCl. Full line curves in the plots are polynomial fits. Concentrations of Ca^{2+} were the same as in Figure 1.

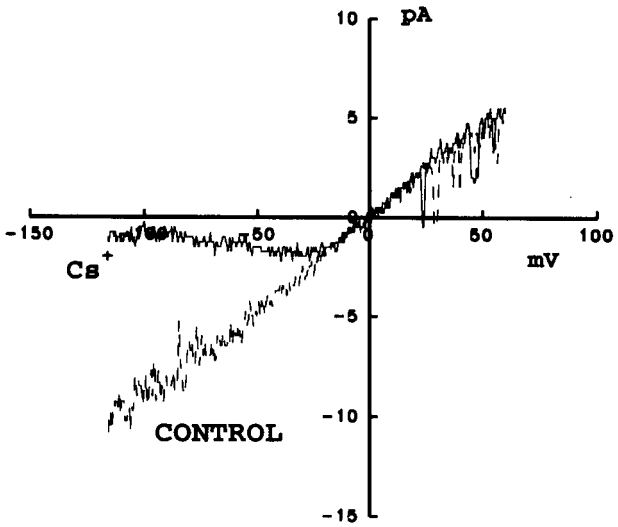

Figure 5. Cs block. Ramp-pulsed recordings (−120 to +60 mV, 500 ms) on an outside-out patch in the absence (CONTROL, interrupted line) and presence (Cs^+, full line) of 10 mM CsCl in the bath. Concentrations of Ca^{2+} and KCl were the same as in Figure 1.

nal TEA, with $K_{½}$ = 1.7 mM compared to 30 mM for the Ca^{2+}-activated K^+ channel. The SDR $K_{½}$ value is in good agreement with 2.3 mM obtained for TEA block in the 100-pS channel found in the *Chara corallina* droplet membrane (Homble et al., 1987). Furthermore, the latter channel showed an inward rectification in the drop-attached mode, with the average conductance of approximately 35 pS at depolarizing potentials, which is quite similar to the behavior of the channel studied here (Fig. 3a).

Tyerman and Findlay (1989) also observed in *C. corallina* droplets a channel with a similar voltage dependence and a conductance of 60 pS, which was believed to be K^+ selective. They also described another K^+ channel type with a conductance of 100 pS. The lower conductance of these two K channel types in *C. corallina* as compared to the values obtained in this study could be explained as a consequence of lower cytoplasmic K^+ concentration in their cell-attached preparation (approximately 70 mM compared to 150 mM in our experiments). It is unlikely that this difference was due to the variation of K^+ channel properties in various charophyte species (at least for the large conductance K^+ channel in different species of *Chara*, the value of 170 pS in symmetrical 150 mM KCl is most often reported [Lühring, 1986; Laver and Walker, 1987; Bertl, 1989]). Interestingly, Bisson et al. (1989) found a 100-pS channel in the droplet membrane of salt-tolerant *Chara buckellii*, which has a low K^+ to Na^+ selectivity ratio (=2.5), which is comparable to the moderate selectivity of the SDR channel. A K^+ channel with conductance (75 pS) and P_{Na}/P_K ratio (0.2) similar to the ones in SDR has also been reported for cytoplasmic droplets in *N. obtusa* (Katsuhara et al., 1991). Therefore, it could be concluded that the K^+ channel investigated here is in fact an obligatory component of the droplet membrane in various characean species.

The TEA sensitivity of the present channel could serve well as an identification marker, since its $K_{½}$ value is 20 times lower compared to the large conductance K^+ channel. The Cs^+ blockade of 90- and 170-pS K^+ channels in *C. gymnophylla* droplets showed a common pattern, although the former channel had relatively higher affinities for both external and internal Cs^+ (compare this study to that of Pottosin, 1992). Moreover, the voltage dependence of the Cs^+ blockade found here resembled even more closely the behavior of K^+ channels of other origin, such as the DR in squid or the Ca^{2+}-activated K^+ channel in smooth muscle (French and Shoukimas, 1985; Cecchi et al., 1987). Although distinguishing poorly among various K^+ channels, Cs^+ is an ideal probe for judging the presence of a specific K^+-selective pore, thus discriminating K^+ channels from other cation channels that could also conduct K^+ ions. This test was necessary for characterization of SDR since it showed only moderate selectivity to alkali cations (based on bi-ionic equilibria).

Voltage Gating

The 90-pS K^+ channel identified here as SDR could probably account for the so-called "early inward current" observed by Homble (1987) in whole-droplet configuration at hyperpolarizing voltages. The decay kinetics of the current through SDR (Fig. 2b) agrees well with the kinetics of this previously reported macroscopic current (Homble, 1987). Moreover, our experiments on whole droplets and outside-out patches containing many 90-pS channels revealed the same kinetics pattern of activation and deactivation of total current in these preparations. Thus, depolarization-activated K^+ channels were likely to dominate the whole-droplet conductance at low cytosolic Ca^{2+} and in the voltage range of −100 to +100 mV (I.I. Pottosin and P.R. Andjus, unpublished data). The depolarization-activated K^+ channel (SDR) studied here showed a characteristic delay in activation, reminiscent of the DR class, previously described in plasma membranes of many animal and higher plant cells (for review, see Schroeder and Hedrich, 1989; Tester, 1990; Blatt, 1991; Hille, 1992). The DR channels have a moderate selectivity compared to

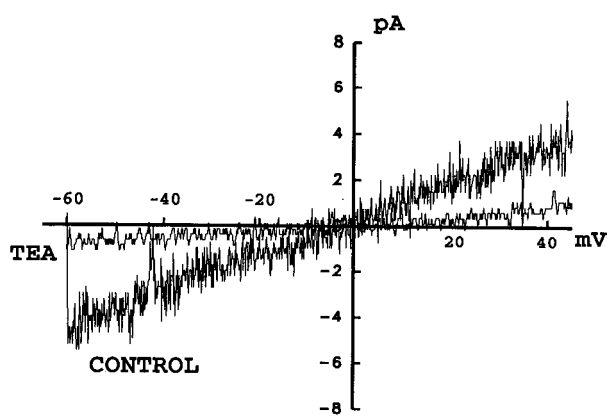

Figure 6. TEA block. Ramp-pulsed recordings (−60 to +45 mV, 500 ms) on an outside-out patch in the absence (CONTROL) and presence (TEA) of 5 mM TEA. The recordings were "digitally filtered" by a running average procedure for n = 3. Concentrations of Ca^{2+} and KCl were the same as in Figure 1.

other K^+ channel types (Latorre and Miller, 1983; Schroeder, 1988). However, the selectivity of a typical DR channel is, nevertheless, higher, and its conductance (5–50 pS) is lower compared to SDR. The latter channel also displayed much slower kinetics of activation, in the range of seconds compared to 10^{-3} s for the DR channel in squid axon (Hille, 1992) or to 10^{-1} s in the outward rectifier in guard cell protoplasts and in the plasmalemma of the alga *Hydrodictyon* (Findlay and Coleman, 1983; Schroeder, 1989). It should be noted, however, that the repolarization of plasmalemma in *Chara* after the action potential spike occurs slowly (within seconds) and is suggested to be due to the function of outwardly rectifying K^+ channels that dominate the resting membrane conductance in the "K state" (Bielby, 1986; Tester, 1988a, 1988b). Accordingly, the K^+ current and the late phase of the action potential in *Chara* showed similar TEA sensitivities (Tester, 1988a) comparable to the TEA sensitivity in SDR.

Origin and Tentative Role of the Channel

The vacuolar perfusion technique used here to obtain droplets assures the tonoplast origin of the bounding membrane with minimal contamination from the plasmalemma. However, the possibility cannot be excluded that the droplets could also be partly bound by newly formed membranes. Similarly, new channels in the droplet membrane may be formed from cytosolic precursors (Alexandrov and Alexandrova, 1990; Tester, 1990). These precursors could be for tonoplast and plasmalemma channels as well. Therefore, to check for the origin of SDR its characteristics were compared to other known channels from both membranes.

The gating kinetics of SDR (i.e. delayed activation at depolarization and slow closure at hyperpolarization) resembled closely the behavior of another slow-gating 100-pS K^+-selective channel in the *Acetabularia* droplet membrane purported to originate from the plasma membrane (Bertl and Gradmann, 1987; Bertl et al., 1988; cf. figs. 10 and 11 in Bertl et al., 1988, with Fig. 2 in this paper). Since the *Acetabularia* droplet membrane is reported to be of plasmalemma origin (Bertl and Gradmann, 1987), the SDR channel could be a component of the same membrane in *Chara*, with the role in membrane repolarization after excitation. However, in this case it is necessary to assume that the voltage dependence of the K^+ current is shifted to more negative potentials (Bielby, 1986; Blatt, 1991) in the diluted environment of the intact cell (0.1–1.0 mM K^+, compared to 150 mM K^+ in our experiments).

On the other hand, there is substantial evidence that the droplet membrane in *Chara* is at least partially made of tonoplast (Lüthring, 1986; Sakano and Tazawa, 1986; Bertl, 1989). The most frequently detected channel type in tonoplasts of various plant tissues shares some properties with SDR: comparable conductance, slow kinetics, and voltage dependence (Hedrich et al., 1986; 1988; Hedrich and Neher, 1987). But this tonoplast channel weakly discriminated between cations and anions (Hedrich et al., 1986; Tester, 1990), was activated by micromolar cytosolic Ca^{2+} (Hedrich and Neher, 1987), and was mainly sensitive to anion channel blockers, although it showed weak sensitivity to TEA (Hedrich and Kurkdjian, 1988). Therefore, that channel and SDR are unlikely to be related. Nevertheless, the existence of specific channel type(s) in the characean tonoplast is not excluded since this membrane also showed electrical excitation, although impaired by the generation of an action potential in the plasmalemma (Lunevsky et al., 1983). The potentials at cytoplasmic sides of both membranes are positively shifted in the course of excitation; hence, their repolarization could be caused by the function of similar outward rectifying K^+ channels.

CONCLUSIONS

The 90-pS K^+-selective and depolarization-activated channel was identified in the droplet membrane of *C. gymnophylla*. This channel shared some properties with the K^+ DRs found in plant and animal cells, albeit having much slower kinetics than in known DR channels. The present SDR channel could be distinguished from the previously described large conductance K^+ channel in the same membrane by lacking Ca^{2+} activation and by having different conductance, selectivity, and gating characteristics and different sensitivity to blockers.

ACKNOWLEDGMENTS

We thank Prof. D. Vučelić, Belgrade University, for the support of this project, and Mrs. Olga Popović for her qualified technical assistance.

Received January 13, 1994; accepted May 30, 1994.

LITERATURE CITED

Alexandrov AA, Alexandrova LA, Berestovsky GN (1990) Block of Ca-channel from algae cells reconstituted in planar lipid bilayer by verapamil. Stud Biophys **138:** 115–118

Bertl A (1989) Current-voltage relationships of sodium-sensitive potassium channel in the tonoplast of *Chara corallina*. J Membr Biol **109:** 9–19

Bertl A, Gradmann D (1987) Current-voltage relationship of potassium channels in the plasmalemma of *Acetabularia*. J Membr Biol **99:** 41–49

Bertl A, Kleiber HG, Gradmann D (1988) Slow kinetics of a potassium channel in *Acetabularia*. J Membr Biol **102:** 141–152

Bielby MJ (1986) Factors controling the K^+ conductance in *Chara*. J Membr Biol **93:** 187–193

Bisson M, Tyerman SD, Findlay GP (1989) Patch clamp studies of ion channels in the membrane of salt-tolerant alga *Chara buckellii* (abstracy No. 270). Plant Physiol **89:** S-45

Blatt MR (1991) Ion channel gating in plants: physiological implications and integration for stomatal function. J Membr Biol **124:** 95–112

Cecchi X, Wolff D, Alvarez A, Latorre R (1987) Mechanisms of Cs^+ blockade in a Ca^{2+}-activated K^+ channel from smooth muscle. Biophys J **52:** 707–716

Findlay GP, Coleman HA (1983) Potassium channels in the membrane of *Hydrodictyon africanum*. J Membr Biol **75:** 241–251

French RJ, Shoukimas JJ (1985) An ion's view of the potassium channel: the structure of the permeation pathway as sensed by a variety of blocking ions. J Gen Physiol **85:** 669–698

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflugers Arch **391:** 85–100

Hedrich R, Barbier-Brygoo H, Felle H, Flügge UI, Luttge U, Maathuis FJM, Mark S, Prins HBA, Raschke K, Schnabl H, Schroieder JI, Struve I, Taiz L, Zeigler P (1988) General mecha-

nisms for solute transport across the tonoplast of plant vacuoles: a patch-clamp survey of ion channels and proton pumps. Bot Acta **101:** 7–13

Hedrich R, Flügge UI, Fernandez JM (1986) Patch-clamp studies of ion transport in isolated plant vacuoles. FEBS Lett **204:** 228–232

Hedrich R, Kurkdjian A (1988) Characterization of an anion-permeable channel from sugar beet vacuoles: effect of inhibitors. EMBO J **7:** 3661–3666

Hedrich R, Neher E (1987) Cytoplasmic calcium regulates voltage-dependent ion channels in plant vacuoles. Nature **329:** 833–836

Hille B (1992) Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA, pp 75–76

Homble F (1987) A tight-seal whole cell study of the voltage-dependent gating mechanisms of K^+-channels of protoplasmic droplets of *Chara corallina*. Plant Physiol **84:** 433–437

Homble F, Ferrier JM, Dainty J (1987) Voltage-dependent K^+-channel in protoplasmic droplets of *Chara corallina*. A single channel patch-clamp study. Plant Physiol **83:** 53–57

Katsuhara M, Mimura T, Tazawa M (1991) Patch-clamp study on ion channels in the tonoplast of *Nitellopsis obtusa*. Plant Cell Physiol **32:** 179–184

Latorre R, Miller C (1983) Conduction and selectivity in potassium channels. J Membr Biol **71:** 11–30

Laver DR, Walker NA (1987) Steady-state voltage-dependent gating and conduction kinetics of single K^+ channels in the membrane of cytoplasmic drops of *Chara australis*. J Membr Biol **100:** 31–42

Laver DR, Walker NA (1991) Activation by Ca^{2+} and block by divalent ions of the K^+ channel in the membrane of cytoplasmic drops from *Chara australis*. J Membr Biol **120:** 131–139

Lühring H (1986) Recording of single K^+ channels in the membrane of cytoplasmic drop of *Chara australis*. Protoplasma **133:** 19–28

Lunevsky VZ, Zhlerova OM, Vostrikov IY, Berstovsky GN (1983) Excitation of Characeae cell membranes as a result of activation of calcium and chloride channels. J Membr Biol **72:** 43–58

Pottosin II (1990) Voltage- and Ca^{2+}-regulated K^+ channel in tonoplast of characean algae. Stud Biophys **138:** 119–126

Pottosin II (1992) Probing of pore in the *Chara gymnophylla* K^+ channel by blocking cations and by streaming potential measurements. FEBS Lett **298:** 253–256

Pottosin II, Andjus PR, Vučelić D, Berestovsky GN (1993) Effects of D_2O on the permeation and gating in the Ca^{2+}-activated potassium channel from *Chara*. J Membr Biol **136:** 113–124

Sakano K, Tazawa M (1986) Tonoplast origin of the envelope membrane of cytoplasmic droplets prepared from *Chara* internodal cells. Protoplasma **131:** 247–249

Schroeder JI (1988) K^+ transport properties of K^+ channels in the plasma membrane of *Vicia faba* guard cells. J Gen Physiol **92:** 667–683

Schroeder JI (1989) Quantitative analysis of outward rectifying K^+ channel currents in guard cell protoplasts from *Vicia faba*. J Membr Biol **107:** 229–235

Schroeder JI, Hedrich R (1989) Involvement of ion channels and active transport in osmoregulation and signalling of higher plant cells. Trends Biochem Sci **14:** 187–192

Tester M (1988a) Blockade of potassium channels in the plasmalemma of *Chara corallina* by tetraethylammonium, Ba, Na and Cs. J Membr Biol **105:** 77–85

Tester M (1988b) Potassium channels in the plasmalemma of *Chara corallina* are multi-ion pores: voltage-dependent blockade by Cs and anomalous permeabilities. J Membr Biol **105:** 159–169

Tester M (1990) Plant ion channels: whole-cell and single-channel studies. New Phytol **114:** 305–340

Tyerman SD, Findlay GP (1989) Current-voltage curves of single Cl channels which coexist with two types of K channel in the tonoplast of *Chara corallina*. J Exp Bot **40:** 105–117

Woodhull AM (1973) Ionic blockade of sodium channels in nerve. J Gen Physiol **61:** 687–708