pH control of the plant outwardly-rectifying potassium channel SKOR

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Received 24 November 1999; received in revised form 28 December 1999

Edited by Pierre Jolles

Abstract SKOR, an *Arabidopsis* depolarisation-activated K^+-selective channel, was expressed in *Xenopus* oocytes, and external and internal pH effects were analysed. Internal pH was manipulated by injections of alkaline or acidic solutions or by acid load from acetate-containing medium. An internal pH decrease from 7.4 to 7.2 induced a strong (ca. 80%) voltage-independent decrease of the macroscopic SKOR current, the macroscopic gating parameters and the single channel conductance remained unchanged. An external acidification from 7.4 to 6.4 had similar effects. It is proposed that pH changes regulate the number of channels available for activation. Sensitivity of SKOR activity to pH in the physiological range suggests that internal and external pH play a role in the regulation of K^+ secretion into the xylem sap.

Key words: K^+ channel; pH; *Xenopus* oocyte; Patch clamp; *Arabidopsis thaliana*

1. Introduction

In *Arabidopsis*, several voltage-gated K^+ channels structurally related to the shaker K^+ channel cloned from *Drosophila melanogaster* [1] have been cloned [2]. They display six putative transmembrane segments (S1 to S6), including a highly charged S4 segment acting as a voltage sensor, and, between S5 and S6, the highly conserved P-domain believed to form part of the pore. Unlike most of their animal counterparts, the plant shaker channels cloned so far do not display inactivation and therefore they can drive sustained K^+ transport. Example of hyperpolarisation-activated plant shaker channels are AKT1 [3,4] and KAT1 [5,6] which allow K^+ influx from the soil solution to the root cells [7] and K^+ influx into guard cells [8], respectively. Up to now, only one channel from the depolarisation-activated plant shaker channel group has been cloned: SKOR, which has been recently demonstrated to be involved in K^+ release into the xylem sap [9]. As the guard cell outwardly-rectifying K^+ channel (Kout) displays functional features reminiscent of SKOR [9,10], it probably belongs to this group.

Besides regulation by membrane potential, the activity of these channels is modulated by other factors, e.g. cyclic nucleotides [4] or phosphorylation [11] and also by the proton concentration in the apoplast as well as in the cytosol [12].

The pH in the cytosol (pHi) as well as in the apoplast (pHe) are known to be influenced by many endogenous compounds and physiological stimuli [13,14]. The role of protons as cytoplasmic second messengers is now well established, particularly in guard cells [15] and in response to plant microorganism interaction [16–18]. In response to these stimuli, increase in pHi is usually accompanied by an increase in K^+ efflux, whereas decrease in pHi is accompanied by decrease in K^+ efflux [19]. In several systems, it has been shown that these changes in K^+ flux result from modulation of K^+ channel activity [12]. For example, pHe and pHi effects on the guard cell Kout of *Vicia faba* have been studied at the single channel level [20,21]. These two reports show that acidification decrease the Kout current without affecting the single channel conductance.

Cloning of plant potassium channels has enabled the analysis of the functional properties in heterologous systems and their sensitivity to pH. For example, it has been shown that K^+ current through the inwardly-rectifying KAT1 channel is increased by internal and external acidification. This modulation involves a shift in KAT1 activation potential toward a less negative membrane potential, without any effect in the single channel conductance [22] (and references therein). Conversely, it was reported that the AKT3 current inhibition by extracellular acidification would originate from a decrease in single channel conductance [23].

Here, pH effect on SKOR macroscopic and single channel currents are analysed and discussed for their physiological significance as well as for their possible relevance to guard cell Kout regulation.

2. Materials and methods

2.1. Expression in Xenopus oocytes

SKOR cRNA [9] was injected into *Xenopus* (CRBM, CNRS, France) oocytes (0.03 μl of cRNA solution containing ca. 1 μg cRNA ml^{-1} per oocyte). Control oocytes were injected with 0.03 μl of deionised water. A home-made temporised pneumatic injector was used for all injections including injections of acid and alkaline solutions during current recording.

2.2. Two-electrode voltage clamp

Macroscopic currents were recorded using the two-electrode voltage-clamp technique, 3 to 7 days after injection as previously described [24]. Solutions used are described in the figure legends.

2.3. Intracellular pH measurements

Intracellular pH was monitored using pH-sensitive microelectrodes prepared and used as described in [25]. Briefly, the interior of the microelectrode was salinised by *N*,*N*-dimethyltrimethylsilylamine vapor (Fluka, Buchs, Switzerland). A column of H^+ cocktail (Hydrogen Ionophore II cocktail A, Fluka) of ca. 300 μm in length, was established at the tip of the microelectrode. The microelectrode was then back-filled with a solution of 300 mM KCl buffered with 10 mM Tris/MES to pH 6.0. Only electrodes that showed linear slopes >55 mV/pH unit change over the calibration range and stable calibration before and after penetration were used. Signals were recorded with an electrometer (Axoprobe, Axon Instruments). On the basis of the calibration curve for the pH-sensitive microelectrodes, the pHi of the oocyte was calculated from the difference between the membrane po-

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PII: S0014-5793(00)01093-0

tential measured simultaneously with a 3 M KCl microelectrode and the potential of the pH-sensitive microelectrode.

2.4. Single channel recording

For patch-clamp experiments, the oocyte vitelline membrane was carefully removed with thin forceps. A devitellinised oocyte was placed in a bath solution containing 100 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES adjusted to pH 7.4 with NaOH; in this solution the resting membrane potential is close to 0 mV. Sylgarded and fire polished pipettes (1 to 3 MΩ) were filled with the 'K10' solution: 10 mM KCl, 90 mM NaCl, 2 mM $MgCl_2$, 10 mM HEPES adjusted to pH 7.4 with NaOH. The patch-clamp amplifier was an Axopatch 200A (Axon Instruments Inc.) interfaced with a TL1 DMA Interface (Axon Instruments Inc.) to a IBM PC-compatible computer. Voltage-pulse protocol application and data acquisition were performed using pClamp (Axon Instruments Inc.) and data analyses using Winascd (G. Droogmans, University of Leuven, Belgium) and Sigmaplot (Jandel Scientific, Erkrath, Germany).

3. Results

3.1. Effect of pH on macroscopic currents

Injection of an acidic or alkaline solution causes a change in the oocyte intracellular pH [26]. Fig. 1A shows normalised current traces elicited by a test pulse of +40 mV on a SKOR expressing oocyte before and after injection of 30 nl of solutions at pH 11 and 3. Injection of the alkaline solution induced a 20% current increase, and injection of the acidic one induced a 30% current decrease. To investigate the regulation of SKOR by pHi, the acid-loading (acetate) method was used [27] and the pHi shift was monitored with a pH microelectrode [25]. The mean pHi value in a standard extracellular solution containing 100 mM Cl^- was 7.4 (7.4 ± 0.05; mean ± S.E.M., $n = 3$ oocytes), consistent with previous reports [25]. Replacing extracellular Cl^- by acetate led, in few minutes, to an intracellular acidification of 0.2 pH unit resulting in a pHi of 7.2 (7.17 ± 0.04; mean ± S.E.M., $n = 3$ oocytes). The internal acidification was found to decrease SKOR K^+ currents (I_{SKOR}) (Fig. 1B) without affecting the macroscopic gating parameters (Fig. 1B, inset). Tail current analysis revealed that the acidification treatment also led to a block of the inward currents (Fig. 1C), without any change in the reversal potential. The inhibition effect was very strong since a 0.2 pHi unit decrease led to a current decrease close to 80% (Fig. 1D). Replacing chloride by malate induced neither a change in pHi nor a change in I_{SKOR} (data not shown).

Acidification of the extracellular medium also inhibited I_{SKOR}. A 7.4 to 6.4 pHe change decreased the current by 50% (Fig. 1D). A stronger external acidification (from 7.4 to 5.6) led to a larger decrease in the outward current (76 ± 2%, mean ± S.E.M., $n = 4$ oocytes). The features of the block were

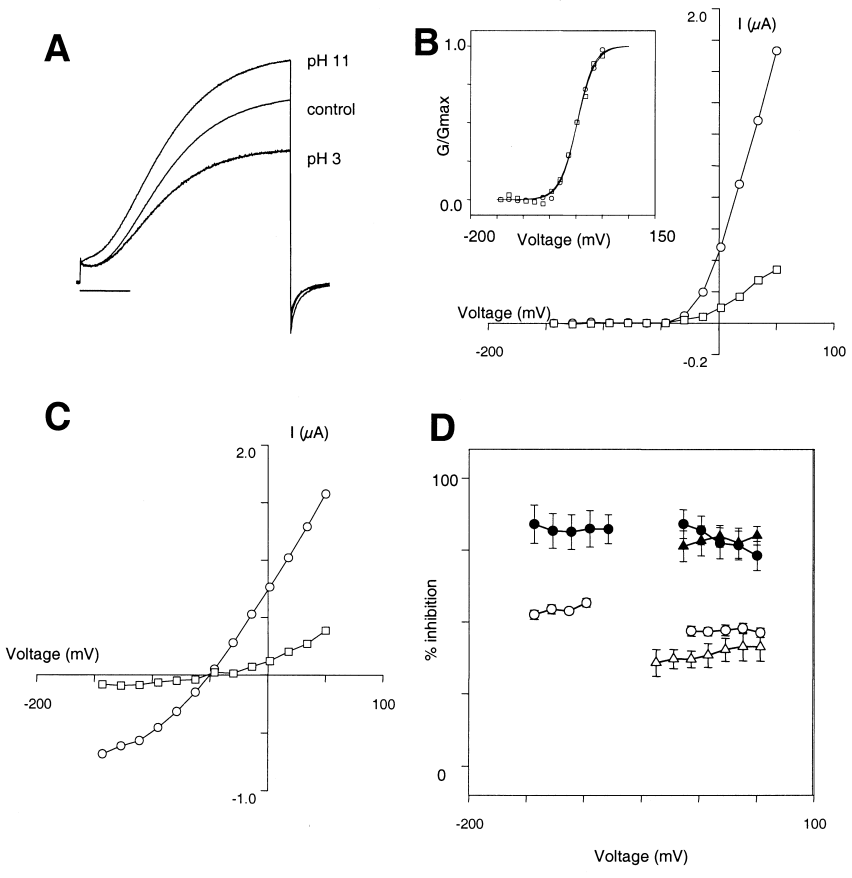

Fig. 1. External and internal acidification decreases SKOR current. A: Effect of intracellular pH change assessed before (middle trace) and after injection of solution containing 100 mM KCl at pH 11 (upper trace) or pH 3 (lower trace). The bathing solution contains 10 mM KCl, 90 mM NaCl, 1 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5 mM HEPES, pH 7.4. B and C: Effect of internal acidification (internal pH was decreased by perfusion of acetate solution, see text) in 10 mM K^+ on steady-state currents (B, inset: relative conductance; pHi = 7.4, open circles and pHi = 7.2, open squares) and initial tail currents (C; pHi = 7.4, open circles and pHi = 7.2, open squares). D: Magnitude of the inhibition of the steady-state current (pHi 7.4 to 7.2, closed triangles; pHe 7.4 to 6.4, open triangles) and of the initial tail current (pHi 7.4 to 7.2, closed circles; pHe 7.4 to 6.4, open circles); results are displayed as mean ± S.E.M. ($n = 5$).

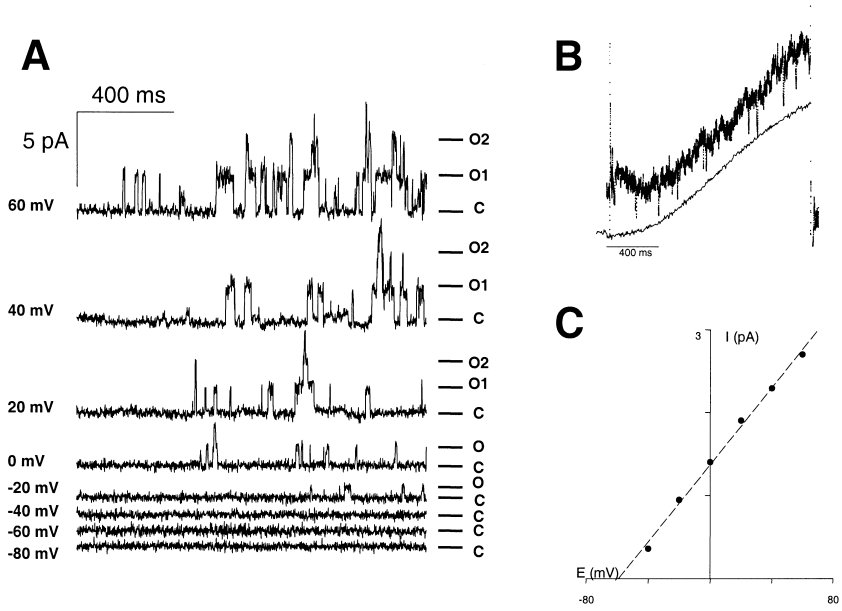

Fig. 2. single channel properties of SKOR. A: Representative single channel currents from SKOR channels recorded in response to depolarisation. The voltages are indicated on the left (C: closed state; O1: one open channel; O2: two open channels). B: Comparison of SKOR activation kinetics obtained by macroscopic currents (two-electrode voltage clamp) and single channel (patch clamp, cell attached). Data points (upper trace) are the means of 200 episodes (lasting 1.5 s each) at +30 mV (from a holding potential of −100 mV). The curve (lower trace) is the normalised macroscopic current recorded at +30 mV with the two electrodes. C: The single open channel current-voltage relationship for SKOR channels over the −80 mV through +80 mV voltage range, pipette K^+ concentration: K10. The *i(V)* curve has been fitted with a linear function, which yields a unitary conductance of 22 pS.

the same as for pHi acidification, without any change in macroscopic gating parameters and in reversal potential (data not shown). These similar effects were not due to a change in pHi, since the oocyte pHi remained stable for several minutes even in a solution at pH 5.5 (checked with use of pH-sensitive microelectrodes).

3.2. Effect of pH on single channel currents

The patch-clamp technique was used on *Xenopus* oocytes to investigate the single channel properties of SKOR. Fig. 2A shows an example of the most frequent single channel activity recorded in membrane patches from SKOR-injected oocytes. At steady state, this activity resulted only in outward currents and was observed only at potentials positive to −60 mV, as previously reported for SKOR macroscopic current [9]. The sum of 200 current records obtained at +30 mV revealed that the single channel current shown in Fig. 2A was indeed mediated by SKOR: the resulting curve was clearly reminiscent of the macroscopic current trace recorded by the two-electrode voltage-clamp technique (Fig. 2B). Analysis of the single channel current-voltage relationship allowed the determination of the single channel conductance (Fig. 2C, Table 1): 23 pS in 10 mM external potassium concentration.

Effects of internal and external acidification were also studied at the single channel level. The SKOR single channel conductance was found to be insensitive to pH change (Table 1). The decrease of macroscopic current cannot therefore be ascribed to a decrease in the single channel conductance.

4. Discussion

4.1. SKOR single channel current is reminiscent of other Kout

These data provide the first characterisation at the single channel level of a cloned shaker-like outwardly-rectifying plant K^+ channel. Interestingly, the SKOR single channel conductance (23 pS in 10 mM external K^+) is similar (taking into account the different K^+ concentration used) to that of Kout characterised in stelar cells of *Arabidopsis* (15.5 pS [28]) where SKOR is expressed [9]. The functional similarities between SKOR and Kout channels characterised in vivo [10,29] suggest that tight structural relationships exist between the various members of the plant family of shaker-like outwardly-rectifying channels. SKOR could therefore constitute a model for studying the structure-function relationship of these channels.

4.2. Mechanism underlying the pH sensitivity

The sigmoidal activation kinetic displayed by I_{SKOR} indicates that the channel has to pass through different closed states (C) before entering the open state (O) [30]: $(C)_n \rightarrow C_1 \rightarrow O$. The exact number of transitions (n+1) cannot be determined from the present data, but the pH effect on the SKOR channel can be discussed with respect to this simple

Table 1
Sensitivity of the single channel conductance (γ) to pH in 10 mM K^+

Solution	pHe = 7.4/pHi = 7.4	pHe = 6.0/pHi = 7.4	pHe = 7.4/pHi = 7.2
γ (pS) mean ± S.E.M.	23.2 ± 0.6	22.7 ± 0.7	20.73 ± 1.21
Number of oocytes	7	6	5

scheme. Protons must act before the $C_1 \rightarrow O$ transition [31,32], since there is no change in gating behaviour (Fig. 1B) and no change in single channel conductance (Table 1). By protonating the channel, acidification (internal or external) would stop the activation scheme before C_1, keeping the channel in a state where it is not available for activation, which could be called a sleepy state [32,33]. Two populations of channels would therefore coexist, those available and those not available for activation, the number of the latter increasing upon internal or external acidification. The fact that a similar conclusion was drawn for the *V. faba* guard cell Kout [20,21] strengthens the hypothesis that the guard cell Kout is a SKOR-like channel (see above).

The similarity (no change in gating behaviour and single channel conductance) of the effects of internal and external acidification on SKOR activity suggests that the mechanisms involved are the same in both cases. This could result from protonation of a site that would be present sometimes at the external face, sometimes at the internal face of the membrane. Interestingly, the S4 transmembrane segment in SKOR bears two histidines at positions equivalent to residues demonstrated to be accessible from both sides of the membrane in the *Drosophila* outwardly-rectifying shaker channel [34]. This hypothesis could be further investigated by site-directed mutagenesis and single channel recordings, as performed for the shaker channel [34].

4.3. pH sensitivity: physiological implications

Previous physiological studies have revealed that translocation of nutrient ions, including K^+, from roots to shoots, is under hormonal control [35]. Downregulation of *SKOR* transcription by ABA [9,36] is likely to take part in this control. However, the rapid decrease in outward channel activity in response to ABA reported in maize stelar cells [37] could not result from such a transcriptional regulation, since it occurs within as little as 2 min of ABA application. It is tempting to speculate that this inhibition is pH mediated: the ABA treatment would induce a decrease in cytosolic pH, leading to inhibition of SKOR activity (since this channel is 80% blocked by internal acidification from 7.4 to 7.2). ABA is known to induce cytosol alkalinisation in guard cells (upregulating outward channel activity [38]). However, it could have an opposite effect in other cells/tissues, since cytosol acidification has been reported in intact *Arabidopsis* seedlings [39].

Acknowledgements: B.L. is endebted to Prof. R. Horn (Jefferson Medical College, Philadelphia, USA) and Prof. F. Bezanilla (University of California, Los Angeles, USA) for helpful discussions. We are very grateful to Dr C. Plassard (INRA Montpellier, France) for the pH microelectrodes, to Dr A.A. Véry and Dr T. Tranbarger for critical reading of the manuscript and to J. Boucherez for expert technical assistance. This work was partly supported by European Communities' BIOTECH Programme (BIO4-CT96) and Rhône-Poulenc.

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