

SHORT COMMUNICATION

Selectivity of the fast activating vacuolar cation channel¹

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Abstract

The FV channel dominates the ion conductance of the vacuolar membrane at physiological Ca²⁺ concentrations. Patch-clamp measurements on whole barley (*Hordeum vulgare*) mesophyll vacuoles and on excised tonoplast patches showed small differences in a selectivity sequence NH₄⁺ > K⁺ ≥ Rb⁺ ≥ Cs⁺ > Na⁺ > Li⁺. Less permeant cations decreased the open probability. The FV channel allows the uptake of small monovalent cations especially NH₄⁺ into the vacuole.

Key words: *Hordeum vulgare*, patch-clamp, vacuolar membrane, cation channel, selectivity.

Introduction

There are two major ion currents across the plant vacuolar membrane, the so-called slow-activating (SV) and fast-activating (FV) vacuolar currents, which are mediated by the SV and the FV channel, respectively. In contrast to the SV channel, the FV channel is active at physiological cytosolic free Ca²⁺ and is blocked at increasing (> 1 μM) cytosolic free Ca²⁺ (Hedrich and Neher, 1987; Allen and Sanders, 1996; Tikhonova *et al.*, 1997). Due to its voltage dependence and Ca²⁺ dependence the FV channel could mediate a rather high K⁺ permeability of the tonoplast, clamping the tonoplast potential close to the equilibrium potential for K⁺ (Tikhonova *et al.*, 1997). This high open probability under physiological conditions raises questions about the selectivity since the FV channel must not interfere with ion compartmentalization into the vacuole. The FV channel has a high selectivity for K⁺ over Cl⁻ and it does not conduct Ca²⁺ (Tikhonova *et al.*, 1997), thus Cl⁻ or Ca²⁺ gradients across the tonoplast can not be discharged by the FV channel. But what about other monovalent cations besides K⁺? Is the

FV channel responsible for the high Rb⁺ permeability of the tonoplast (MacRobbie, 1995)? Does the FV channel conduct Na⁺, which under salinity should be compartmentalized inside the vacuole? To answer these questions, the selectivity of the FV channel was studied in barley mesophyll vacuoles at the single channel level as well as with whole vacuoles.

Materials and methods

Barley (*Hordeum vulgare* L.) mesophyll protoplasts were isolated as described (Tikhonova *et al.*, 1997). A strong suction pulse applied with a patch-microelectrode ruptured the plasma membrane of a single selected protoplast to release a vacuole. The standard bath solution contained (in mM): 100 KCl, 5 EGTA, 15 TRICINE/TRIS (pH 7.5). Patch-clamp microelectrodes were filled with the same solution for measurements with excised membrane patches, and for whole vacuole measurements with (in mM): 100 KCl, 5 EGTA, 15 MES/TRIS (pH 6.5) or 100 KCl, 2 CaCl₂, 15 MES/TRIS (pH 6.5). Different monovalent cations (K⁺, NH₄⁺, Rb⁺, Cs⁺, Na⁺, Li⁺, arginine⁺, and TRIS⁺) were applied by bath perfusion at 100 mM chloride salts. Osmolalities of all solutions were adjusted to 650 mOsm with sorbitol and verified by a cryoscopic osmometer (Osmomat 030, Germany). All experiments were performed under continuous bath perfusion (5–10 μl s⁻¹). Electrophysiological measurements were carried out as described in detail in Tikhonova *et al.* (1997). Vacuolar ion currents were measured under voltage-clamp conditions using three different recording modes (Hamill *et al.*, 1981): whole vacuole (analogous to whole cell), and cytoplasmic side-out (cytoplasmic side of the membrane faces the bath) as well as vacuolar side-out excised membrane patches. The convention of current and voltage was according to Bertl *et al.* (1992), i.e. the sign of voltage refers to the cytosolic side, and positive (outward) currents represent cation flux from the bath into the vacuole.

Results

To record large instantaneously activating whole vacuole (FV) currents at positive and negative potentials, Ca²⁺

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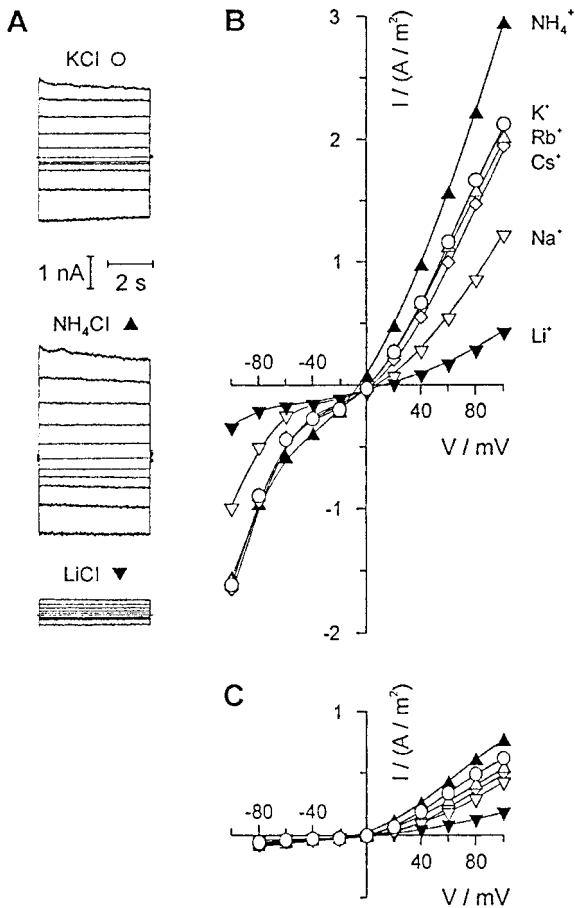


Fig. 1. Whole vacuole currents with different monovalent cations (as indicated) on the cytosolic side of the vacuolar membrane. From a holding potential of 0 mV the electrical potential was changed for 5 s in 20 mV steps from -100 or -80 to +100 mV. (A) Original whole vacuole currents (I in nA) in symmetrical low Ca²⁺ (<1 nM). (B) Corresponding whole vacuole I/V relationships (n=3, SD in the range of symbol size). Current amplitudes were normalized to the vacuolar membrane surface and expressed in A m⁻². Data points were joined by a 4th order spline. (C) Whole vacuole I/V relationships determined in the presence of 2 mM vacuolar Ca²⁺ (symbols as in (B); n=6, SD in the range of symbol size). By bath perfusion different monovalent cations were applied to the same vacuole.

was kept low (<1 nM) on both sides of the vacuolar membrane (Tikhonova *et al.*, 1997). Under these conditions, whole vacuole I/V relationships displayed a characteristic non-linear shape (Fig. 1) due to the voltage-dependent open probability of the FV channel (Tikhonova *et al.*, 1997). Substitution of K⁺ (100 mM) by other monovalent cations on the cytosolic side of the vacuolar membrane resulted in increasing outward currents carried by NH₄⁺ whereas Rb⁺ and Cs⁺ yielded comparable current amplitudes. With Na⁺ and Li⁺ not only outward currents carried by these cation decreased, but also the inward current carried by K⁺ efflux from the vacuole was diminished (Fig. 1). The decrease was more pronounced in the presence of Li⁺. At the end of each experiment, lasting up to 40–50 min, currents were

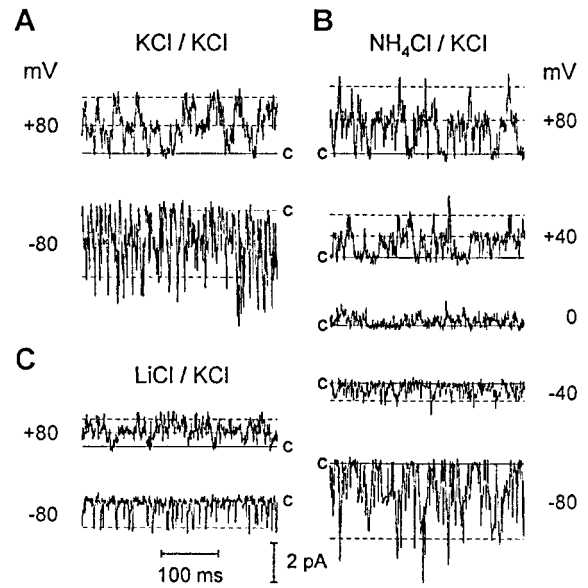


Fig. 2. Single channel currents measured at different electrical potentials (as indicated) with an excised cytoplasmic side-out membrane patch. The closed state of the ion channels (C) is indicated by a solid line and one or two open channels are indicated by dashed lines. The bath solution (cytosolic side) was changed from KCl (A) to NH₄Cl (B), and to LiCl (C), 100 mM each.

measured with K⁺ again. A decrease of current amplitudes by less than 10% was observed between the initial and the final measurement in KCl. The reversal potential (V(I=0)) compared to K⁺ shifted by -6 mV with NH₄⁺, by 0 mV with Rb⁺, by +3 mV with Cs⁺, by +5 mV with Na⁺, and by +10 mV with Li⁺ (Fig. 1B). When the vacuolar Ca²⁺ concentration was increased to 2 mM, to reflect the physiological Ca²⁺ gradient across the vacuolar membrane, inward currents vanished due to a voltage-dependent block by vacuolar Ca²⁺ (Tikhonova *et al.*, 1997). Outward current amplitudes displayed the same order NH₄⁺ > K⁺ ≥ Rb⁺ ≥ Cs⁺ > Na⁺ > Li⁺ as in the absence of vacuolar Ca²⁺ (Fig. 1C). Substitution of K⁺ by arginine or TRIS resulted in no detectable instantaneously activating whole vacuole currents.

Single-channel recordings of the FV channel were carried out with isolated vacuolar side-out membrane patches as well as with cytoplasmic side-out membrane patches (Fig. 2). Measurements were done in the absence of Ca (<1 nM) on both sides of the vacuolar membrane to enable single-channel activity at positive and negative membrane potentials. Comparable to whole vacuole currents, a substitution of K⁺ at the cytosolic side by NH₄⁺ resulted in slightly increased single channel current amplitudes at positive membrane potentials (Figs 2, 3A). A substitution of K⁺ by Li⁺ decreased single channel current amplitudes of the FV channel (Figs 2, 3D). With different cations at the cytosolic side of the vacuolar membrane, single-channel current amplitudes at positive potentials (Fig. 3) showed the same order as the instant-

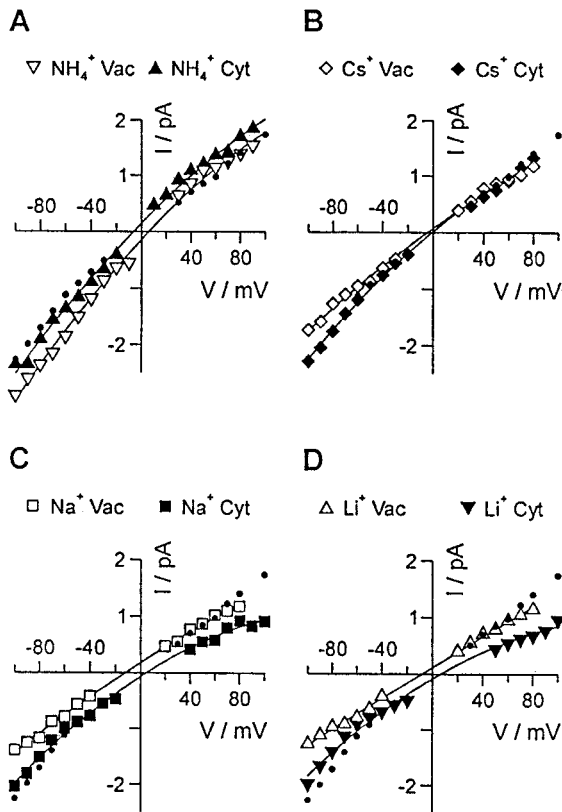


Fig. 3. Single channel I/V relationships under bi-ionic conditions. Starting in symmetrical 100 mM KCl () with vacuolar side-out membrane patches/cytoplasmic side-out membrane patches ($n=3$) the bath solution (vacuolar side/cytosolic side) was changed to NH_4Cl (A), CsCl (B), NaCl (C), and LiCl (D). Data points were joined by 2nd order polynomials.

aneously activating whole vacuole currents (Fig. 1B), namely $\text{NH}_4^+ > \text{K}^+ \geq \text{Rb}^+ \geq \text{Cs}^+ > \text{Na}^+ > \text{Li}^+$. Reversal potentials of open FV channel I/V relationships under bi-ionic conditions (Fig. 3) were comparable to reversal potentials of whole vacuole I/V relationships indicating a permeability sequence of $\text{NH}_4^+ > \text{K}^+ \geq \text{Rb}^+ \geq \text{Cs}^+ > \text{Na}^+ > \text{Li}^+$. With vacuolar side-out membrane patches substitution of K^+ by different monovalent cations shifted the reversal potential in the opposite direction compared to cytoplasmic side-out membrane patches (Fig. 3) indicating the same permeability sequence.

Discussion

All six monovalent cations tested here, namely NH_4^+ , K^+ , Rb^+ , Cs^+ , Na^+ , and Li^+ , permeate the FV channel. The relative permeability among these cations varies little. Reversal potentials of whole vacuole (Fig. 1) and open FV channel I/V relationships (Fig. 3) according to the GHK equation (Hille, 1992) indicate a 25% higher permeability for NH_4^+ compared to K^+ . Rb^+ and Cs^+ had a permeability comparable to K^+ , and Na^+ and Li^+ showed a 20% and a 30% smaller permeability, respectively. The

permeability sequence $\text{K}^+ \geq \text{Rb}^+ \geq \text{Cs}^+ > \text{Na}^+ > \text{Li}^+$ of the FV channel corresponds to Eisenmann sequence III, indicating a 'weak site', i.e. the binding of cations to the FV channel is mainly determined by dehydration energies (Hille, 1992). The FV channel has a high selectivity for K^+ over Cl^- , and the divalent cation Ca^{2+} blocks FV channels at micromolar concentrations (Tikhonova *et al.*, 1997). Summarizing, the FV channel is a cation channel, which is selectively permeable for small monovalent cations, but discriminates little among them.

Compared to this, the slow-activating vacuolar (SV) channel is permeable for both mono- and divalent cations (Ward and Schroeder, 1994; Pottosin *et al.*, 1997). The electrophysiological behaviour of plant plasma membranes seems to be dominated in most cases by highly selective K^+ channels (Schroeder, 1988; Hedrich and Dietrich, 1996). In the plasma membrane of endosperm cells a non-selective cation channel with selectivity comparable to the FV channel has been described (Stoeckel and Takeda, 1989). Ca^{2+} , however, activates this channel (Stoeckel and Takeda, 1989). For the plasma membrane from rye (*Secale cereale*) roots a cation channel has been reported, which is voltage-independent and not affected by Ca^{2+} , but has a selectivity comparable to the FV channel (White and Tester, 1992; White, 1996).

A quantitative comparison shows that whole vacuole current amplitudes (Fig. 1) were affected by different cations on the cytosolic side to a larger degree than single channel current amplitudes (Fig. 3). Compared to this, the voltage-dependence and the sensitivity towards inhibitors like Ca^{2+} or different polyamines is the same for single FV channels and whole vacuole currents measured in the absence of Ca^{2+} (Tikhonova *et al.*, 1997; Brüggemann *et al.*, 1998). This indicates that under the measuring conditions used here, whole vacuole currents are due to the activity of FV channels only. At +80 mV substitution of K^+ by NH_4^+ increased whole vacuole currents by 33% whereas single channel current amplitudes increased by 21% only. Substitution by Na^+ or Li^+ decreased whole vacuole currents by 49% or 83% whereas single channel current amplitudes decreased by 35% or 52%, respectively. This shows that NH_4^+ , Na^+ , and Li^+ affect the gating behaviour of the FV channel. NH_4^+ increases the open probability, and Na^+ or Li^+ decrease the open probability. The latter is especially obvious from the decreased whole vacuole currents at negative membrane potentials. Cytosolic Na^+ or Li^+ suppresses these currents which are carried by K^+ , in a voltage-independent manner. The different whole vacuole current amplitudes observed with different monovalent cations (Fig. 1), are due to different single channel conductances (Fig. 3) and to an activation of the FV channel by NH_4^+ and an inhibition by Na^+ or Li^+ , respectively.

What is the physiological role of the FV channel? At physiological cytosolic Ca^{2+} activities (200 nM) the FV

channel has a high open probability (Hedrich and Neher, 1987; Allen and Sanders, 1996) and may thus determine the membrane potential of the vacuolar membrane (Tikhonova *et al.*, 1997). According to the data presented here the FV channel conducts all small monovalent cations. Since K^+ is the dominant alkali cation in the cytosol as well as inside the vacuole, the membrane potential of the vacuolar membrane should come close to the Nernst potential for K^+ . This is in good agreement with available data (Bethmann *et al.*, 1995). NH_4^+ , which slightly activated the FV channel and resulted in the largest single channel currents (Fig. 3A) is expected to permeate the vacuolar membrane rapidly. This is in good agreement with earlier measurements indicating a high NH_4^+ permeability of the vacuolar membrane. This gives rise to uptake of non-assimilated NH_4^+ into the vacuole (Macklon *et al.*, 1990) resulting in vacuolar NH_4^+ concentrations slightly below (Wang *et al.*, 1993) or above the cytosolic NH_4^+ concentration (Lee and Ratcliffe, 1991). Also the high Rb^+ permeability of the tonoplast observed in flux experiments with isolated guard cells (MacRobbie, 1995) is likely to be caused by open Rb^+ -permeable FV channels. At physiological vacuolar Ca^{2+} activities, ranging from 200 μM (Bethmann *et al.*, 1995) to 2.3 mM (Felle, 1988), the FV channel is outwardly rectifying (Fig. 1) due to the voltage-dependent block of the FV channel by vacuolar Ca^{2+} (Tikhonova *et al.*, 1997). Thus the FV channel will easily allow cations to enter the vacuole, but cation release is less likely. Especially Na^+ , which additionally blocks the FV channel, is compartmentalized inside the vacuole under salt stress, and must not be released into the cytosol. The FV channel is a pathway that allows the passive uptake of small monovalent cations from the cytosol into the vacuole.

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