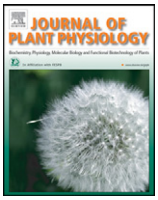
Journal of Plant Physiology 167 (2010) 1566–1570

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph



Cadmium and selenium modulate slow vacuolar channels in rape (*Brassica napus*) vacuoles

Halina Dziubinska[a], Maria Filek[b,c], Elzbieta Krol[a], Kazimierz Trebacz[a,*]

[a] *Department of Biophysics, Institute of Biology, Maria Curie-Skłodowska University, Akademicka 19, PL-20-033 Lublin, Poland*
[b] *Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, PL-30-239 Kraków, Poland*
[c] *Institute of Biology, Pedagogical University, Podbrzezie 3, 30-084 Kraków, Poland*

ARTICLE INFO

Article history:
Received 2 March 2010
Received in revised form 11 May 2010
Accepted 2 June 2010

Keywords:
Brassica napus
Cadmium
Selenium
SV channels
Vacuole

ABSTRACT

Currents flowing through slow vacuolar SV channels of rape (*Brassica napus*) growing on media supplemented with Cd^{2+} (400 μM), and/or SeO_4^{2-} (2 μM) were examined. The aim of the study was to investigate the role of Cd^{2+} in modulation of SV channel activity and to determine whether Se reverses the effect of cadmium. Vacuoles were isolated using a quick surgical method to avoid application of any cell wall-degrading enzymes. Vacuoles of rape exhibited typical SV channel activity with slow activation at positive potentials and strong rectification into the vacuolar lumen. Single-channel conductance in cytoplasm-side-out tonoplast patches ranged between 68.8 ± 1.9 pS in the control, 80.1 ± 2.5 pS, in Cd^{2+}-, 74.2 ± 2.4 pS in Cd^{2+}/selenate-, and 80.1 ± 1.8 pS in selenate-pretreated plants. The lack of a clear tendency was likely an effect of equilibration of the pipette solution (without Cd^{2+}/SeO_4^{2-}) with that of the luminal side of the vesicles. In the vacuole-attached configuration, in which natural vacuolar solution was not exchanged, there was a significant reduction in single-channel conductance in the Cd^{2+}- (40.3 ± 2.8 pS), Cd^{2+}/selenate- (47.1 ± 2.8 pS) and selenate-pretreated (42.3 ± 1.4 pS) plants, compared to the control (60.2 ± 1.7 pS). The reduction in single-channel conductance only partially explained the significant decline in the densities of ion current flowing through the vacuolar membrane in the whole-vacuole configuration in the plants growing on Cd^{2+} and Cd^{2+}/selenate media. Thus, Cd^{2+} accumulation in the vacuole reduced the number of active SV channels from 0.28 ± 0.05 μm^{-2} in the control to 0.021 ± 0.005 and 0.039 ± 0.004 μm^{-2} in Cd^{2+} and Cd^{2+}/selenate-pretreated plants, respectively.

Introduction

Cadmium is found in the environment as a main metal pollutant toxic to many plants and animals. In living organisms, the mechanisms of cadmium action are multifarious. Primarily because of its bioavailability, cadmium mimics other metals that are essential for diverse biological functions (Martelli et al., 2006). Physiological effects and toxicity of metals strongly depend on their intracellular localization and binding to organelles and ligands (Sokolova et al., 2005). One strategy to avoid their harmful effects is to keep the concentration of toxic metal ions in the cytoplasm low by preventing the metal from being accumulated within the cell, either by increased binding of metal ions to the cell wall or by reduced uptake through cation/proton exchangers, or by pumping the metal out of the cell with active efflux pumps, a mechanism that is widespread in metal-tolerant bacteria (Tong et al., 2004). The other strategy is to detoxify heavy metal ions entering the cytoplasm through inactivation via chelation or conversion of a toxic ion into a less toxic or easier to handle form, and/or compartmentalization.

The vacuole is generally considered to be the main storage site for metals in yeast and plant cells (Hirschi, 2001). Compartmentalization of metals in the vacuole is also a part of the tolerance mechanism of some metal hyperaccumulators.

The calcium level is known to be one of the factors affecting the degree of cadmium tolerance in plants (Antosiewicz and Hennig, 2004). From the outside of cells, cadmium can alter the intracellular concentration of calcium, which is a universal and versatile intracellular signal messenger (Berridge et al., 2000). The ionic radii of cadmium and calcium are almost identical (0.97 and 0.99 Å, respectively) and it has been suggested that Cd^{2+} ions can be transported via Ca^{2+}-channels (Pedersen and Bjerregaard, 2000; Bondgaard and Bjerregaard, 2005). In neurons, cadmium transport via voltage-gated channels was indicated both in the absence of external calcium and in the presence of calcium (Gavazzo et al., 2005). However, this metal is able to cross the plasma membrane even after the blockage of voltage-gated calcium channels, indicat-

---

Abbreviations: *I*/*V*, current/voltage characteristics; MS, Murashige and Skoog medium; SV, slowly activating vacuolar channels.

[*] Corresponding author. Tel.: +48 81 537 59 31; fax: +48 81 537 59 01.
E-mail address: kazimierz.trebacz@poczta.umcs.lublin.pl (K. Trebacz).

0176-1617/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2010.06.016

ing that the cells possess other means of cadmium entry.

The aim of the present experiments was to investigate the possibility of involving vacuolar ion channels in response to cadmium treatment in rape plants. On the basis of our earlier study, it was suggested that Cd action on rape cells could be connected with its influence on ion transporters or with its binding to metallothioneins (Filek et al., 2008). Additionally, selenoproteins, which contain l-selenomethionine and l-selenocysteine residues, have been detected and implicated in cellular protection systems against heavy metals (Low and Berry, 1996; El-Sharaky et al., 2007; Filek et al., 2008). The protective role of selenium observed in these studies could be a consequence of a selenium-confined removal of Cd^{2+} from metabolically active cellular sites. Cadmium ions have a high affinity to thiol groups and easily form cadmium–thiol complexes (Vairavamurthy et al., 2000). Thus, changes in the vacuolar channel activity in both the cadmium- and selenium-treated plants were investigated here.

Slowly activating vacuolar channels (SV) were chosen for our experiments. SV channels are the most frequently examined plant vacuolar ion channels (Allen and Sanders, 1997; Pottosin and Schönknecht, 2007). SV channels are abundant in vascular and some lower plants (Trebacz et al., 2007). SV channel proteins identified as TPC1 (Peiter et al., 2005) are highly expressed in vacuoles. Based on their surface density of approx. 0.1–1 channel per μm^2 (Pottosin and Schönknecht, 2007), it is possible to estimate the number of active SV channel copies per vacuole as 1000–30,000. The channels are permeable to potassium and to divalent cations, including Ca^{2+} (Pottosin et al., 2001, 2004). SV channels exhibit slow kinetics of activation at positive membrane potentials. Current saturation occurs within up to 1 s. Patch-clamp experiments revealed that, at symmetric ion concentrations, the channels are strongly rectifying and pass cations preferentially from the cytosol to the vacuole. However, for most physiologically relevant cations, including Ca^{2+} and metal pollutants like Cd^{2+}, the electrochemical potential gradient is directed to the opposite direction – from the vacuole to the cytoplasm (Pottosin and Schönknecht, 2007). SV channels are activated by the cytoplasmic calcium ions at the micromolar range (Hedrich and Neher, 1987). Addition of Mg^{2+} lowers the Ca^{2+} threshold of SV channel activation (Pei et al., 1999). The precise role of SV channels in cellular processes has not yet been established. However, their modulation by a variety of factors is well documented. The known SV channel-modifying agents include: 14-3-3 proteins, nickel, zinc, calmodulin, kinases and phosphatases, pH, reducing and oxidizing agents, and polyamines (Davies and Sanders, 1995; Bethke and Jones, 1997; Carpaneto et al., 1999, 2001; Dobrovinskaya et al., 1999; Pei et al., 1999; Schulz-Lessdorf and Hedrich, 1995; van den Wijngaard et al., 2001; Wherrett et al., 2005). Such broad susceptibility to different regulatory factors makes SV channels important in cell signaling. In this study, we examined the possible regulatory roles of Cd and Se ions in the activity of these abundant ion channels.

Material and methods

Plant material

Seeds of winter rape (*Brassica napus* L. var. Górczański) were sterilized in 70% ethanol for 3 min, in 3.1% NaOCl for 15 min, and then rinsed three times with sterile water. Next, the seeds were transferred into sterile Magenta vessels and placed on MS media (Murashige and Skoog, 1962) containing mineral components, 3% sucrose, 0.6% agar, pH 5.8 (control) and on MS media with Cd, Se and Cd + Se content. Cadmium sulphate ($CdSO_4$, Sigma–Aldrich) was used at a 400 μM concentration and sodium selenate (Na_2SeO_4, Sigma–Aldrich) at a concentration of 2 μM. 2-week-old seedlings cultured at 20 °C, with a 16 h/8 h (day/night) photoperiod (150 μmol quantum m^{-2} s^{-1}) were chosen to investigate the activity of vacuolar channels.

Isolation of vacuoles

The vacuoles of rape were isolated using the method described by Trebacz and Schönknecht (2000). Fragments of leaves were plasmolysed in a medium containing: 100 mM KCl, 2 mM $CaCl_2$, 15 mM Hepes/Tris, pH 7.2, 500 mM sorbitol. After 1 h incubation in the plasmolysing medium, the leaf fragments were cut with a sharp razor blade. Protoplasts were liberated through incised cell walls during a stepwise deplasmolysis. Reduction of the osmolality of the perfusion solution to 300 mOsm/kg caused rupturing of protoplasts and isolation of vacuoles.

Patch-clamp experiments

The patch-clamp experiments were performed as described previously (Trebacz and Schönknecht, 2000; Trebacz et al., 2007). Three basic configurations of the patch-clamp method were applied: “vacuole-attached”, “whole-vacuole” and “cytoplasm-side-out” (Hamill et al., 1981). An Ag/AgCl reference electrode was connected to the bath solution through a salt bridge filled with 100 mM KCl. Microelectrodes were pulled from borosilicate glass capillaries Kimax 51 (Kimble Products, USA) with the micropipette puller PP-830 (Narishige, Japan). The tips were fire-polished by a microforge MF 200-2 (World Precision Instruments, USA). The micropipettes were filled with a filtered solution containing 100 mM KCl, 2 mM $CaCl_2$, 15 mM Mes/Tris, pH 5.85 immediately before each experiment. The standard bath solution contained: 100 mM KCl, 2 mM $CaCl_2$, 15 mM Hepes/Tris, pH 7.2. Osmolality of all solutions was adjusted with sorbitol to 300 mOsm/kg under the control of cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Patch-clamp amplifier EPC-9 (Heka Elektronik, Germany) running under PULSE (Heka Elektronik, Germany) was used for data registration and acquisition. The convention of current and voltage signs was according to Bertl et al. (1992). It implies that positive current represents a cation flux from the cytosol to the vacuole. SV current density was obtained by dividing ion current by the surface area of the vacuole. Ion channel density was determined by dividing the whole-vacuole current density at +100 mV by the single-channel ion current in outside-out configuration extrapolated to +100 mV. Registration of ion currents was performed in darkness to avoid light susceptibility of selenium.

Results

The primary aim of this study was to evaluate the effect of cadmium accumulation in rape vacuoles on the activity of the abundant SV channels. Given that selenium partially reverses the inhibitory effect of cadmium, vacuoles growing in the medium containing both elements were also examined. In addition to the untreated plants, vacuoles from rape plants pretreated with selenium alone were used as another control.

In our previous study (Dziubinska et al., 2008), we showed that rape leaf vacuoles possess typical SV channels. The current passing through the channels is activated in the presence of Ca^{2+} ions on the cytosolic side of the vacuolar membrane. Fig. 1 presents traces of ion currents flowing through single open SV channels in membrane patches isolated from rape leaf vacuoles. Characteristic kinetics of SV currents were observed, with clear single-channel openings at negative potentials and time-dependent activity at the beginning of positive potential clamping. To avoid uncertainty in determination of single-channel openings at positive potentials, only ion currents at negative voltages were chosen for the analysis. Fig. 2