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Effects of Cu^{2+} on characteristic of SV currents

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Abstract Applying the patch-clamp technique to vacuoles from Radish we studied the effects of Cu^{2+} on Slow Vacuolar (SV) current's characteristic. Our results show that Cu^{2+} in bath solution at higher concentration inhibits SV currents and the percentage of inhibition increases with increasing concentration and changes with different voltage. When at lower concentration, Cu^{2+} significantly promotes the SV currents and the promotion ratio decrease with increasing voltage. At the same time, the time constants of activation become lesser after adding Cu^{2+}. These results show that there may be some Cu^{2+} binding sites on SV channels and binding to which can change SV current's characteristic.

Keywords: the patch-clamp technique, vacuolar, Cu^{2+}, SV channel.

Vacuole is an important component of mature plant cells, epiphytes and algae (except for prokaryocytes). It is not only a store of metabolites and inorganic ions avoiding poison, but also a multifunctional organelle which adjusts the metabolism of cells; controls compounding, accumulation, and transportation of many substances; maintains stabilization inside conditions of cell; mitigates effects of environment and enhancing viability[1]. Investigations have identified that the ion channels and pumps are the pathways of the movement of ions and metabolites[2].

Ion channels play very important roles in the growth development and the process of cell responding to the outside[3]. The Slow Vacuolar (SV) channel is universal in tonoplast and cation selective. Voltage- and time-dependent SV channels, as well as vacuolar K^+ selective (VK) channels, are activated by cytosolic Ca^{2+}[4,5].

Copper, an abundant transition metal in soils, is an essential micronutrient of plants and participates in many responses of metabolism in plants, but absorbing too much copper is toxic. Toxic concentrations of Cu^{2+} inhibit metabolic activity, which leads to suppressed growth and slow development[6]. Too much copper ion influence permeability of plasma membrane through raise non-selectivity conductance and repress the proton pump (H^+-ATPase) activity[7]. Moreover, a great deal of copper will break plasmalemma, resulting in leakage of cytosolic ion[8] and metabolize maladjustment of water[9]. It is one of the mechanisms that too much copper results in poisoning and harming to the plants[10]. However, the relevant study of copper in domestic is much concentrated on the research of distribution of geographic situation, investigation on category and measurement of the content. However, little is known about how Cu^{2+} affects electricity physiology and ion channels by patch-clamp.

We have made some preliminary study on the influence of the copper ion on SV current before. The result showed that $CuCl_2$ at a lower concentration (about < 5.5×10^{-5} mol·L^{-1}) promoted SV currents and at higher concentration (about > 5.5×10^{-5} mol·L^{-1}) it inhibited SV currents[11]. However, it did not show the effect on the promotion ratio, inhibition ratio, time constant of activate channel and their relations with concentration and voltage. In the present study the effects of $CuCl_2$ on the Characteristic of SV currents are showed. It obtained a series of result, such as the following: when adding $CuCl_2$ with the low concentration in bath solution, the promotion ratio on SV current reduces along with the increase of voltage. At the same time, the time constant of activation decreases obviously when the membrane potential is between 20—120 mV, but at more than 120 mV, the change is not obvious; when at higher concentration, the inhibition ratio on SV current enhances with the increase of $CuCl_2$ concentration and changes with different voltage (enlarge first and let up again). These results indicate that there may be some Cu^{2+} binding sites on SV channel, which can change some characteristics of the channel after binding Cu^{2+} and affects the signal conduction, and thus directly or indirectly affect the growth of the plants.

1 Experimental

1.1 Reagents and preparation of experimental solutions

Reagents: KCl, $CaCl_2$, $MgCl_2$ and $CuCl_2$ were obtained from Beijing Chemical Reagent Company. HEPES, EGTA, MES, Tris-base and *D*-sorbitol were obtained from Sigma Company.

The standard solutions used in patch-clamp experiments were composed of 100 mmol/L KCl, 2 mmol/L $MgCl_2$, 5 mmol/L MES-Tris, pH = 5.5 in pipette solutions (luminal side), and 100 mmol/L KCl, 10 mmol/L HEPES-Tris, pH = 7.3 in bath solutions (cytosolic side)[5]. The respective concentration of $CaCl_2$ and $CuCl_2$ that was added into pipette solutions and bath solutions will be expounded later in this paper. Osmolalities of pipette solutions and bath solutions were adjusted to 460 and 390 mmol/kg respectively by the addition of *D*-sorbitol.

1.2 Isolation of radish vacuoles

Radish vacuoles were isolated from fresh taproot grown in the field[2,5]. A slice of storage tissue was cut off with a razor blade and the surface was rinsed with some drops of bath solution to wash the extruded vacuoles directly into the recording chamber.

1.3 Patch-clamp experiments

Patch-clamp pipettes were prepared from soft glass capillaries (BJ-40, Beijing) and pulled on a multi-stage programmable puller. Patch pipettes had a tip resistance of 5—7 MΩ when filled with pipettes solution. Followed by the formation of a Giga-Ω seals between electrode and the vacuolar membrane (above 10 GΩ), the patch was disrupted by suction to obtain the whole-vacuolar clamp configuration at 20—25℃[12]. The vacuoles were voltage-clamped by using CV203BU HEADSTAGE (Axon Instruments), the currents were amplified by using an amplifier (Digidata 1200), and the data were acquired by using the software PCLAMP 6.0. The data were analyzed and figures were plotted with Clampfit (Axon Instruments) and software MICROCAL-ORIGN (5.0). The time constants (τ) of activation obtained by fitting the SV current by a monoexponential function.

2 Results

2.1 SV channel currents

We recorded the SV channel currents of radish vacuoles respectively at 0, 1, 2 mmol·L^{-1} $CaCl_2$ in bath solution. Membrane potential was stepped from –40 to +180 mV with 20 mV increments from a holding potential of 0 mV. The episode duration was 4.1 s. Bath solutions contained 100 mmol/L KCl, 10 mmol/L HEPES-Tris, and pH =7.3. Pipette solutions were composed of 100 mmol/L KCl, 2 mmol/L $MgCl_2$, 5 mmol/L MES-Tris, with pH 5.5. The same experiments were repeated (n>5 vacuoles) and the results were similar, but there was a tiny difference in currents. Fig. 1 showed the SV current of 1 mmol·L^{-1} $CaCl_2$ in bath solution. The current-voltage relationship of different concentration of $CaCl_2$ performed in Fig. 2.

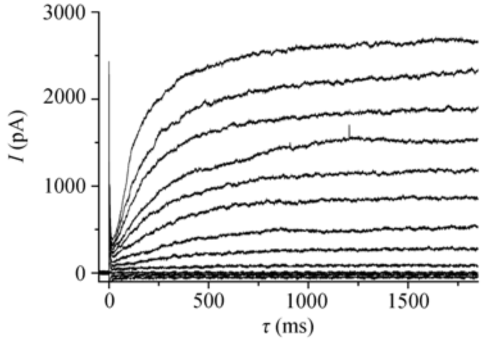

Fig. 1. Voltage-dependent SV channel.

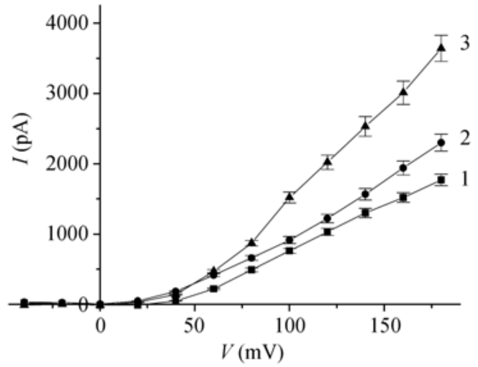

Fig. 2. Current-voltage curve of $CaCl_2$ at different concentration in the bath solutions (0 mmol·L^{-1} (1), 1 mmol·L^{-1}(2), 2 mmol·L^{-1}(3)).

2.2 *Effect of $CuCl_2$ at different concentrations in the bath solutions on SV channel currents*

Membrane potential was stepped from –40 to +180 mV with 20 mV increments from a holding potential of 0 mV, and the episode duration was 4.1 s. Bath solutions contained 100 mmol/L KCl, 2 mmol/L $CaCl_2$, 10 mmol/L HEPES-Tris, and pH =7.3, and different lower concentration $CuCl_2$ of 0, 1×10^{-8}, 1×10^{-7} 1×10^{-6} mol·L^{-1} (respectively corresponding of control, Cu1, Cu2, Cu3). Pipette solutions were composed of 100 mmol/L KCl, 2 mmol/L $MgCl_2$, 5 mmol/L MES-Tris, with pH 5.5. We recorded SV current. At each concentration, the data were the average of those recorded for at least 5 separate vacuoles. After contrasted to control, lower concentration $CuCl_2$ increased SV currents (Fig. 3(a)) and improved the permeability of tonoplast. Our previous study[11] showed that the maximum concentration of $CuCl_2$ improved SV currents was about 1×10^{-7} mol·L^{-1}, but at more concentration, increase of currents became lesser (Fig. 3(a)). The promotion ratio was calculated by dividing the increase value of the SV current by the current of control. Fig. 3(b) showed promotion ratio-voltage curve of lower concentration of Cu^{2+}. The curve was very steep between 20 and 60 mV, and the slope dramatically decreased at more positive potentials (Fig. 3(b)).

When at the higher concentration of $CuCl_2$ in the bath solution (Cu4: 1×10^{-4} mol·L^{-1}, Cu5: 5×10^{-4} mol·L^{-1}), it inhibited SV currents (Fig. 4(a)). The inhibition ratio was calculated by dividing the decrease value of the SV current by the current of control. Fig. 4(b) showed inhibition ratio-voltage curve of higher concentration of Cu^{2+}. The inhibition ratio increased with the increasing of $CuCl_2$ concentration and changed with different voltages (increased first and decreased again along with the increase of voltage) (Fig. 4(b)).

2.3 *Effect of $CuCl_2$ on time constant of activation of SV channel*

Time constant (τ) was obtained by fitting the SV currents by a monoexponential function. Fig. 5

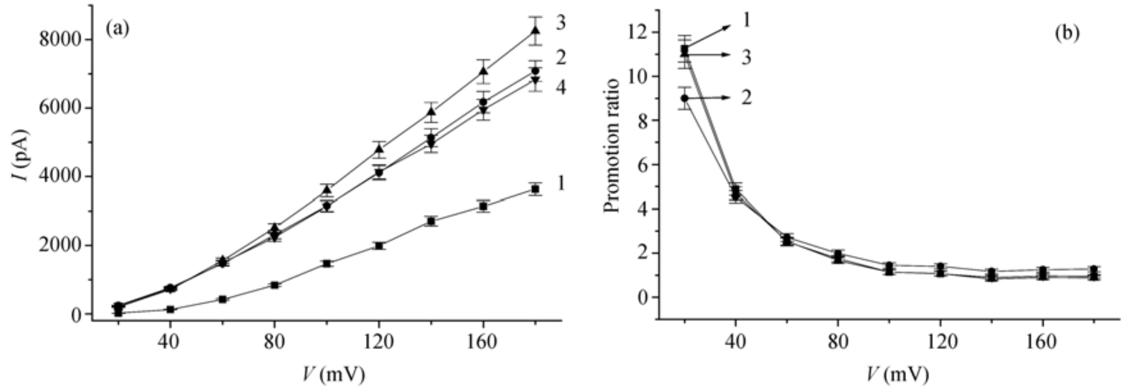

Fig. 3. Effects of $CuCl_2$ at lower concentration in the bath solutions on SV currents. (a) Current-voltage curve (control(1), Cu1(2), Cu2(3), Cu3(4)); (b) promotion ratio-voltage curve of $CaCl_2$ (Cu1(1), Cu2(2), Cu3(3)).

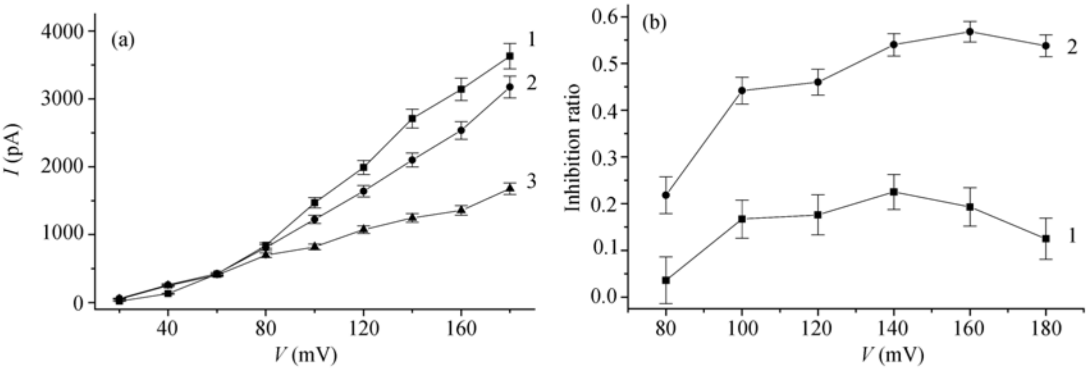

Fig. 4. Effects of $CuCl_2$ at higher concentration in the bath solutions on SV currents. (a) Current-voltage curve (control (1), Cu4 (2), Cu5 (3)); (b) inhibition ratio-voltage curve of $CaCl_2$ (Cu4 (1), Cu5 (2)).

showed τ-voltage curve of control and lower concentration $CuCl_2$. In contrast, time constants were decreased when a lower concentration Cu^{2+} was added into the bath solutions (Fig. 5). The curve was relatively steep between 20 and 120 mV, and the slope dramatically decreased at more positive potentials. At the same time, time constant reduced remarkably along with the voltage increase of all curves.

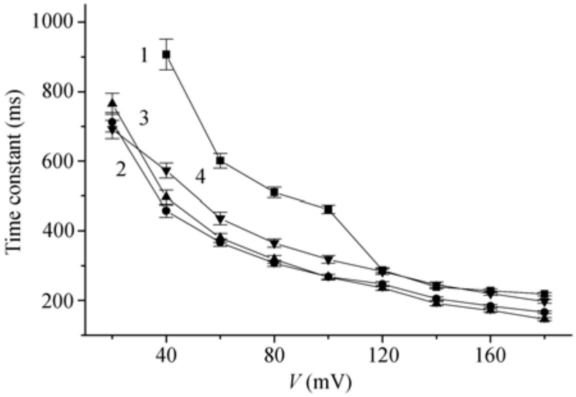

Fig. 5. Effects of $CuCl_2$ at lower concentration in the bath solutions on time constant (control (1), Cu1 (2), Cu2 (3), Cu3 (4)).

3 Discussion and conclusion

Vacuolar is the storage of high plants and maintains normal physiological function of the plants through storing the surplus composition in the nutrient and the metabolism abundance and releasing in time when the plant lacks certain nourishment. The SV channel is the first vacuolar ion channel to be discovered[13] and plays a key role in regulating concentration of cytosolic cations by storing and releasing. Many cations containing monovalent cations (K^+, Na^+, Cs^+) and divalent cations (Mg^{2+}, Ca^{2+}, Ba^{2+}) can penetrate SV channel[5]. Ca^{2+}, as the second messenger, regulates the cell multi-function and responds in time to stimulation of environment through changing concentration and causing a series of reactions of the cells. Therefore, SV channel plays very important roles in maintaining the cell's normal function.

In this study, we investigated the effects of $CuCl_2$ on SV current's characteristic. Lower concentration $CuCl_2$ increased SV currents and improved the permeability of tonoplast. The promotion ratio decreased quickly at low voltage and slowly at high voltage, while the time constant reduced obviously along with voltage increasing and remarkably between 20 and 120 mV. These results show that there may be some Cu^{2+} binding sites on the SV channel protein and binding to which can bring about the deformation of protein conformation, which increases the permeability of vacuolar membrane. At the same time, decreasing time constant can improve activation of channel and quicken the response to stimulation of environment, which can enhance anti-adversity function of the plant cell. However, at higher concentration, Cu^{2+} inhibits the SV current, and the inhibition ratio increases with increasing Cu^{2+} concentration. It is deduced that the action mechanism of this process of higher Cu^{2+} may compete with Ca^{2+} for binding site of activation SV channel and lead to decreasing SV current. These indicate that Cu^{2+} may affect directly development of plants by changing current and progress of channel activation or indirectly by second messenger of Ca^{2+} changing. These results provide a powerful proof on the channel's level for researching further the effects of Cu^{2+} on the physiology of plants and the influence of copper on farm crops.

The similar result was found as Ba^{2+} at low concentrations promotes the SV currents while at high concentration inhibits the SV currents[14]. What is the relation between these results and the most suitable nourishment laws (the plant cannot live when it lacks a certain essential chemical element, when that chemical element is just the right amount the plant can grow healthily, but too many and this element may turn harmful[15]) was put forward by French scientist G. Bertrand. However, the mechanism of this kind of special effect is still not entirely clear and must be researched further. The research on the action mechanism of metal ions to the physiology of plants is a very complicated process, which needs to be demonstrated and perfected from different angles and with many different methods.

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