The effect of Ca^{2+} concentration on response to salinity stress in *Chara australis*

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It has been known for a long time that higher Ca^{2+} concentration in the medium ameliorates salinity stress (Hoffmann & Bisson, 1988; Shabala *et al.*, 2006). The mechanism of this effect, however, is not clearly understood. We used the charophyte cell system to resolve the effect of Ca^{2+} and high Na^+ on various membrane transporters. Charophytes are the sister plants to ancestors of higher plants. Their large cell size minimises the damage of electrode insertion and the electrical characteristics can be measured for many hours. Charophytes contain both salt-tolerant and salt sensitive genera.

We exposed salt-sensitive *Chara australis* to artificial pond water (APW) with 50 mM Na^+ and two Ca^{2+} concentrations: 0.1mM (high Na^+/low Ca^{2+}) and 1.0mM (high Na^+/high Ca^{2+}). To accustom the cells to lower turgor, they were initially exposed to sorbitol APW with osmolarity equivalent to that of 50mM NaCl. The membrane potential difference (PD) was voltage-clamped to bipolar staircase command to obtain current/voltage (I/V) profiles. These were then modelled to resolve the electrical characteristics of the proton pump, the inward and outward rectifiers and the background current (Beilby & Walker, 1996) as function of time in the two high salinity media.

Sorbitol APW. The I/V profiles were very similar to those in APW, despite of reduction of the turgor pressure. This lack of response contrasts with the turgor decrease-activation of the proton pump in the salt-tolerant charophyte *Lamprothamnium succinctum* (Al Khazaaly & Beilby, 2007). The results suggest that the *Chara* turgor sensor is not operating, or that the signal from the sensor to the pump is not communicated.

High Na^+/high Ca^{2+} APW. The resting PD changed from -227 ± 7 mV in sorbitol APW to -184 ± 20 mV. The resting PD exhibited small oscillations of amplitude 0.5 to 1.0mV. The background conductance increased about four times compared to that in APW and sorbitol APW. The channels passing the background current are thought to be like the NSCCs (Non selective cation permeable channels) found in higher plants (Shabala *et al.*, 2006), which provide one of the pathways for Na^+ to enter the cells. In this medium the proton pump exhibited activation, but the increased rate of proton pumping was not sufficient to keep the membrane PD from depolarising. Another response to increased salinity was the inability of the proton pump to withstand voltage clamp to levels far from the resting PD (Beilby, 2007). After voltage-clamps to levels more negative than about -280mV, the pump was inhibited for tens of minutes and recovered very slowly or not at all. This effect limited the span of the I/V characteristics.

High Na^+/low Ca^{2+} APW/ following high Na^+/high Ca^{2+} APW. The surviving cells became even more fragile under voltage-clamp protocols and hyperpolarised limit for the I/V protocol was made less negative at -230 mV. The resting PD depolarised to -150 ± 20 mV. The proton pumping rate declined, below that observed in APW, but the background conductance remained at the high level similar to that in high Na^+/high Ca^{2+} APW. The resting PD exhibited small oscillations 2 to 5mV.

High Na^+/low Ca^{2+} APW/ following sorbitol APW. These cells exhibited greater increase in the background conductance than the group pre-treated in High Na^+/high Ca^{2+} APW. The proton pump was totally inhibited in most of the cells with the resting PD of -100 ± 30 mV. Some cells exhibited repetitive spontaneous action potentials.

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