Modelling ion transporters at the time of hypertonic regulation in Lamprothamnium succinctum (Characeae, Charophyceae)

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The aim of this study was to monitor the electrical characteristics of a plant cell membrane subjected to hypertonic stress and to separate the responses of the different ion transporters to salinity increase and turgor decrease. Charophytes are an ideal system for studying electrophysiology at single cell level, as the large cell size minimises microelectrode injury. Their phylogenetic position, as sister plants to the ancestors of land plants, ensures the general applicability of the data collected.

The whole cell current-voltage (I/V) characteristics of the salt-tolerant charophyte Lamprothamnium succinctum (A. Braun in Ascherson) R.D. Wood were measured for up to 7 h following the hypertonic challenge. The I/V profiles were modelled to resolve the roles of the various transporters. To distinguish between the responses to increase in salinity and to decrease in turgor, cells were exposed to a step from ⅓ to 1/6 artificial seawater (ASW) and to equivalent osmotic increase by addition of sorbitol (Sorbitol ASW). In each case we observed the membrane potential difference (PD) becoming transiently more negative. The steady state membrane PD of cells in 1/6 ASW was -133 ± 10 mV (9 cells). The maximum membrane PD in ⅓ ASW of -145 ± 10 mV (7 cells) was reached in 110 ± 91 minutes after the start of the hypertonic stress. The control cells for the Sorbitol ASW experiments were in K⁺ state with membrane PD of -96 ± 10 mV (9 cells) in 1/6 ASW. The maximum membrane PD in Sorbitol ASW of -151 ± 10 mV (5 cells) was reached after 148 ± 74 minutes.

The modelling was implemented by fitting the Two State HGSS (Hansen, Gradmann, Sanders and Slayman) model to the proton pump. The GHK (Goldmann, Hodgkin and Katz) model supplemented by the Boltzmann distribution was employed to fit the large conductance K⁺ channels, dominating the membrane conductance in the K⁺ state, and the inward and outward rectifiers. An empirical model was fitted to the background current. Modelling resolved the hypertonic response to changes in the proton pump, background conductance, the half-activation potential and sometimes the gating charge of the inward rectifier. The pump model parameters \( k_0 \) and \( \kappa_0 \) (in s⁻¹) increased from 4500 and 180 in ⅓ ASW to 5000 and 850 in 1/6 ASW, respectively. The background conductance increased from 6.3 S/m² in ⅓ ASW to 9.6 S/m² in 1/6 ASW. The half-activation PD for the inward rectifier depolarised from -330 to -310 mV. In Sorbitol ASW the pump was activated with maximal \( k_0 \) and \( \kappa_0 \) (in s⁻¹) of 35000 and 55. The background conductance decreased from 2.75 S/m² in ⅓ ASW to 2.0 S/m² in Sorbitol ASW. The half-activation PD for the inward rectifier depolarised from -380 to -355 mV. Therefore, the turgor decrease alone initiated the response of the inward rectifier and the proton pump. The increase in salinity was responsible for the rise in the background conductance.

The responses of the transporters as function of time in ⅓ ASW and Sorbitol ASW were modelled, starting with the control in either pump state or K⁺ state. While there was a great variability from cell to cell in the timing of the different transporters, the inward rectifier activation at more positive PDs preceded the maximum pump activation.

The experiments described here are a part of detailed analysis of the salt tolerance mechanisms at the single cell level. The identity of the salinity stress detector and the signalling cascades activating the regulatory ion transporters are unknown in any plant system. Using charophytes as an experimental system provides another advantage of comparison between salt-tolerant genera (such as Lamprothamnium) and salt-sensitive ones (such as Chara).

Key words: hypertonic turgor regulation; Lamprothamnium; proton pump model; GHK model; background conductance; inward rectifier