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A revised and unified pressure-clamp/relaxation theory for studying plant cell water relations with pressure probes: In-situ determination of cell volume for calculation of volumetric elastic modulus and hydraulic conductivity

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HIGHLIGHTS

- Accurate estimates of plant cell volume (ν_o) can be determined in-situ using cell-pressure-probes.
- A revised ν_o-theory was developed that is valid for the pressure-clamp and pressure-relaxation methods.
- For the same cell, the pressure-clamp method gave a systematically lower (21%) ν_o as compared to the pressure-relaxation method.
- Effects of solute mixing could only explain a potential error in calculated ν_o of $<3\%$.
- The results suggest that both methods are differentially effected by cell osmotic behavior (i.e. solute reflection coefficient, σ) in response to turgor changes.

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ABSTRACT

The cell-pressure-probe is a unique tool to study plant water relations in-situ. Inaccuracy in the estimation of cell volume (ν_o) is the major source of error in the calculation of both cell volumetric elastic modulus (ε) and cell hydraulic conductivity (Lp). Estimates of ν_o and Lp can be obtained with the pressure-clamp (PC) and pressure-relaxation (PR) methods. In theory, both methods should result in comparable ν_o and Lp estimates, but this has not been the case. In this study, the existing ν_o-theories for PC and PR methods were reviewed and clarified. A revised ν_o-theory was developed that is equally valid for the PC and PR methods. The revised theory was used to determine ν_o for two extreme scenarios of solute mixing between the experimental cell and sap in the pressure probe microcapillary. Using a fully automated cell-pressure-probe (ACPP) on leaf epidermal cells of *Tradescantia virginiana*, the validity of the revised theory was tested with experimental data. Calculated ν_o values from both methods were in the range of optically determined ν_o (=1.1–5.0 nL) for *T. virginiana*. However, the PC method produced a systematically lower (21%) calculated ν_o compared to the PR method. Effects of solute mixing could only explain a potential error in calculated ν_o of $<3\%$. For both methods, this discrepancy in ν_o was almost identical to the discrepancy in the measured ratio of $\Delta V/\Delta P$ (total change in microcapillary sap volume *versus* corresponding change in cell turgor) of 19%, which is a fundamental parameter in calculating ν_o. It followed from the revised theory that the ratio of $\Delta V/\Delta P$ was inversely related to the solute reflection coefficient. This highlighted that treating the punctured cell as an ideal osmometer in both methods is potentially not correct. Effects of non-ideal osmotic behavior by transmembrane solute movement may be minimized in the PR as compared to the PC method.

1. Introduction

The cell-pressure-probe has been used for more than 30 years to study water relations in-situ at the individual cell level (Hüsken et al., 1978; Steudle, 1993; Tomos and Leigh, 1999), mostly in higher plants (Tomos et al., 1981; Tyerman and Steudle, 1982; Zhu and Steudle, 1991; Moore and Cosgrove, 1991) but also in algal

Abbreviations: ACPP, automated cell pressure probe; PC, pressure-clamp; PR, pressure-relaxation

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Nomenclature

A	cell membrane surface area (m^2)
b	($\sigma\pi_o$) when using the PC method and ($\varepsilon+\sigma\pi_o$) when using the PR method (MPa)
J_v	water flow across the cell membrane ($m\ s^{-1}$)
J_s	solutes flow across the cell membrane ($mol\ s^{-1}$)
Lp	cell hydraulic conductivity ($m\ s^{-1}\ MPa^{-1}$)
N	amount of cell solutes (mol)
ΔN	change in solutes of the cell compartment via the microcapillary between original and final equilibrium state of the cell in the PC and PR method (mol)
P	cell hydrostatic pressure, measured as gauge pressure (MPa)
ΔP	change in cell hydrostatic pressure between original and final equilibrium state of the cell in the PC and PR method (MPa)
R	ideal gas constant ($m^3\ MPa\ K^{-1}\ mol^{-1}$)
t	time (s)
Δt	time period of application of the PR and PC method (s)
T	temperature (K)
$T_{1/2}$	halftime of relaxation processes in $\Delta P(t)$ or $\Delta V(t)$ (s)
V	cell sap volume in microcapillary (m^3)
ΔV	change in microcapillary sap volume between original and final equilibrium state of the cell in the PC and PR method (m^3)
dP/dV	cell elastic modulus ($MPa\ m^{-3}$)
ε	cell volumetric elastic modulus ($=\nu_o\ (dP/dV)$) ($m^3\ MPa\ m^{-3}$=MPa)
π	cell osmotic pressure (MPa)
ν	cell volume, defined as the volume bounded by the cell membrane, and calculated in (m^3) but expressed in (nL)
σ	solute reflection coefficient (1)
Ψ	cell water potential (MPa)

Subscripts

f	final equilibrium in J_v
i	initial change in microcapillary sap volume corresponding to the induced step change from P_o to P_f in the PC method
m	change in microcapillary sap volume corresponding to the flow of water across the membrane when P is clamped at P_f in the PC method
o	original equilibrium in J_v
t	microcapillary tip
ex	external

(Steudle and Tyerman, 1983; Henzler et al., 2004) and fungal cells (Cosgrove et al., 1987). All of these systems have a large central vacuole, a tough cell wall, and can develop a relatively high hydrostatic turgor pressure (P). Using the cell-pressure-probe a cell is punctured with the tip of a glass microcapillary which causes cell sap volume entering the microcapillary (V). Prior to measuring P, V external to the cell is minimized so that it ideally equals the volume of glass introduced into the cell (i.e. restoration of cell volume). After the cell has reached its equilibrium state, P and V are monitored. Although the microcapillary tip is only in hydraulic contact with the vacuole, both vacuolar and cytoplasmic compartments must exhibit the same P (the unsupported vacuolar membrane cannot support a pressure difference), and hence at Ψ equilibrium the same osmotic pressure (π). Thus, the cell is regarded as an osmotic system having a volume (ν) equivalent to that of the outermost cell membrane. Beside the direct measurement of P, the cell-pressure-probe can artificially impose small short-term changes in both ν and P by small changes in V, which allows the determination of two fundamental cell properties, the cell volumetric elastic modulus (ε) and cell hydraulic conductivity (Lp) (Steudle, 1993). Both parameters depend on original cell volume (ν_o)

$$\varepsilon = \nu_o \frac{dP}{dV} \tag{1}$$

and

$$Lp = \frac{\ln 2\nu_o}{AT_{1/2}b} \tag{2}$$

where in practice, very small and rapid changes in cell sap volume in the microcapillary and the corresponding measured changes in cell turgor are considered equivalent to dV and dP, respectively. When dV/dP is measured using such changes, water flows across the cell membrane are negligible, and therefore dP is proportional to the elastic change in cell volume of $d\nu$ ($=dV$) (i.e., Eq. (1) is analogous to Hooke's law). These rapid changes in cell volume and pressure (dV and dP) should not be confused with ΔV and ΔP which represent changes as measured between the original and final equilibrium state of the cell when subjected to the pressure-clamp (PC) and pressure-relaxation (PR) method (Steudle, 1993; Wendler and Zimmermann, 1982) (Fig. 1). These two different hydraulic methods for manipulating P and V can be used to induce water flows across the cell membrane and to determine Lp of Eq. (2). Mainly due to the difference in cell elastic behavior between both methods, b of Eq. (2) is equivalent to $\sigma\pi_o$ when using the PC method and ($\varepsilon+\sigma\pi_o$) when using the PR method (Wendler and Zimmermann, 1982). Nevertheless, both methods should give equivalent estimates of Lp for the same cell, but this has not been the case. The Lp of parenchyma cells of sugar cane differed by 50% to 59% (Moore and Cosgrove, 1991), and the Lp of wheat root cortical cells by 16% to 21% (Zhang and Tyerman, 1991), between both methods. Recent technical developments have included a computer vision-based automated-cell-pressure-probe (ACPP) system that enables a finer control and higher resolution of P and V changes with limited manual input from the operator (Wong et al., 2009). However, even after incorporating those improvements, Lp of leaf epidermal cells of *Tradescantia virginiana* still differed by 17% to 31% between both methods (Wada et al., 2014). It is not clear what causes this discrepancy in calculated Lp between the PC and PR methods for the same cell.

The biggest source of error in determination of ε and Lp is the estimation of ν_o. Inaccuracy in optical ν_o can be $>50\%$ due to irregularities in cell shape and cell to cell variations (Tomos et al., 1981; Malone and Tomos, 1990). Therefore, theories have been developed independently for the PC (Wendler and Zimmermann, 1982) and PR (Malone and Tomos, 1990) method to derive ν_o directly and in-situ for the punctured cell. This biophysical approach has the advantage that it eliminates errors in estimated ν_o caused by destructive tissue sampling. However, ν_o calculated using these methods and existing theories differs by around 17% for the same cell (Zhang and Tyerman, 1991; Wada et al., 2014). Murphy and Smith (1998) reported that solute mixing between

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