Water channels in Chara corallina

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Abstract

Water relations parameters of Chara corallina internodes were measured using the single cell pressure probe. The effect of mercurials, which are recognized as non-specific water channel inhibitors, was examined. HgCl₂ concentrations greater than 5 mmol m⁻³ were found to inhibit hydraulic conductivity (Lp) close to 90%, whereas pCMPS was found to have no effect on Lp. The activation energy of water flow was increased significantly from 21.0 kJ mol⁻¹ to 45.6 kJ mol⁻¹, following the application of HgCl₂. These results are in accordance with evidence for Hg²⁺-sensitive water channels in the plasma membrane of charophytes (Henzler and Steudle, 1995; Tazawa et al., 1996). The metabolic effects must, however, be considered in view of the rapid inhibition of respiration and the depolarization of the membrane potential with HgCl₂ concentrations lower than those found to affect Lp. It was possible to measure simultaneously water relations and membrane PD, in order to examine the contribution of potassium channels to Lp. Cells were induced into a K⁺ permeable state. The K⁺ channels, assumed to be open, were subsequently blocked by various blockers. No significant difference in Lp was found for any of these treatments. Finally, the permeability of C. corallina membranes to ethanol was examined. HgCl₂ was found to cause a decrease in reflection coefficient, coinciding with a decrease in Lp, but there was no change in the ethanol permeability coefficient. This has been interpreted in terms of both the frictional model and composite model of non-electrolyte membrane transport.

Key words: Water channels, Chara, hydraulic conductivity, membrane transport models, reflection coefficient.

Introduction

Certain membrane integral proteins (MIP) that have been located to the plasma membrane (Kammerloher et al., 1994) and tonoplast (Höfte et al., 1992; Maurel et al., 1993) of plant cells behave as water channels in heterologous expression systems. These proteins are thought to be arranged tetramERICally within lipid bilayers, to form stable, functional water channels (Walz et al., 1994). Mercurial derivatives have been extensively employed in water channel studies, as blockers of water transport by the proteins. The reversible blockage of Hg²⁺ ions has largely been attributed to the cysteine-189 residue of water channel proteins, via cysteine substitution studies (Preston et al., 1993; Zhang et al., 1993; van Os et al., 1994). Further, a mercury-insensitive water channel has recently been cloned, which lacked the cysteine residue (Hasegawa et al., 1994).

The precise molecular pathway of water movement through these channels has yet to be determined. Thermodynamic water relations parameters, namely osmotic permeability (Pₒ), diffusional permeability (P_d) and activation energy (E_a), provide an empirical description of water flow across membranes. Evidence for water channels, based on these parameters, encompasses a high Pₒ value (>10⁻²⁴ m s⁻¹), a PₒP₆ ratio exceeding 1 and an Eₐ value pertaining to the Eₐ of diffusion in bulk solution (Eₐ<25kJ mol⁻¹) (Verkman, 1989, 1992).

The mercurials HgCl₂ and pCMBS (p-chloromercuriphenylsulphonic acid) have been found to effect a
reduction in $P_{os}$, tending to $P_{os} = P_d$, which was reversed in the presence of the reducing agent mercapto ethanol (Meyer and Verkman, 1987).

Although, mercurials have been found to have no effect on the osmotic permeability of artificial lipid bilayers (Benga et al., 1983), their non-specific effect on other sulphhydril containing proteins in cell membranes and on the overall physiology of cells, must be considered.

Giant charophyte cells present a simple model of membrane transport mechanisms in plant cells. Wayne and Tazawa (1990) have implicated differential gating mechanisms of pathways for endosmotic and exosmotic water permeation across *Nitellopsis* cell membranes. Exosmotic $Lp$ was reported to be decreased significantly upon time-dependent exposure to pCMPS, whereas the potassium channel blocker dodecyltrimethylammonium bromide (Dod) was an inhibitor of endosmotic $Lp$. When $\gamma$ TIP was expressed in oocytes the large increase in $Lp$ was not correlated with additional electrogenic ion transport (Maurel et al., 1993), alternatively indicating that there are distinct pathways for ion and water movement across membranes. Tazawa et al. (1996) have recently shown for *Chara corallina*, using the technique of transcellular osmosis, that polarity of water flow was abolished when maximal inhibition of HgCl$_2$ occurred. They conclude that polarity of water flow is a result of intrinsic properties of water channels.

The single cell pressure probe permits direct measurement of a variety of parameters correlated to water and solute flow across biological membranes, where both osmotic and hydrostatic pressure gradients can be utilized (Hüskens et al., 1978). In this study, the pressure probe was used to determine the hydraulic conductivity of *C. corallina* internodes (related to $P_{os}$ by $P_{os}V/wRT$, where $V_w$ is the partial molar volume of water, $R$ is the gas constant and $T$ is absolute temperature). Further, the solute permeability ($P_s$) and the interaction of solutes with the cell membranes, in terms of the reflection coefficient ($\sigma_s$) of solutes, was determined.

Dainty and Ginzburg (1963) developed a model which describes $\sigma_s$ in terms of water and solute movement through aqueous channels:

$$\sigma_s = 1 - \frac{V_s P_s}{LpRT} - \frac{K_s f_{sw}}{f_{sw} + f_{sm}}$$

where $V_s$ is the partial molar volume of the solute; $f_{sw}$ is the frictional interaction between solute and water within a channel; $f_{sm}$ is the frictional interaction of solute with the channel and $K_s$ is equal to the ratio of concentrations of solute in the channel to that in the solution. The last term in the equation defines the solvent drag and membrane drag effect of solutes moving through aqueous channels. A large frictional term should imply significant solvent–solute interaction within water filled pores of the membrane. Equation 1 is derived for the situation in which water flow through the lipid bilayer is insignificant compared to the flow through pores, whereas the solute may move across the lipid bilayer and the pores. This assumption may not be valid when the pores are blocked.

Reflection coefficients have been defined within the limits of zero and unity, describing no membrane selectivity and relative membrane impermeability for a solute, respectively (Dainty and Ginzburg, 1963; Diamond and Wright, 1969). Negative reflection coefficients have been measured in the past (Tyerman and Steudle, 1982), and are possible theoretically, in relation to equation 1. The second term on the right side of the equation is unlikely to be responsible for a negative reflection coefficient (Steudle and Henzler, 1995), and it is more reasonable to expect a large contribution of the third term, namely large frictional forces, to result in a negative measurement of $\sigma$ (Steudle and Tyerman, 1983).

The frictional model presented by equation 1 is based upon the concept of homogeneous membranes. In contrast to this model, Kedem and Katchalsky (1963) have presented a composite membrane model, describing 'patchy' membranes, where distinct patches within the membrane are denoted by distinct and non-additive water relations parameters. Henzler and Steudle (1995) have recently applied this model to describe water and solute transport across *C. corallina* membranes. For a composite membrane, consisting of two parallel arrays/patches (a, b), the reflection coefficient of the whole membrane is described by:

$$\sigma_s = \frac{\gamma_s Lp_a}{Lp} \sigma_a + \frac{\gamma_b Lp_b}{Lp} \sigma_b$$

where $\gamma_s$ and $\gamma_b$ are the fractional contribution of each array, respectively.

The aim of this study was to examine evidence for water channels in *C. corallina* internodes and subsequently to examine the nature of these channels with respect to selectivity and mercury sensitivity. Emphasis has also been put on elucidating the specificity of the mercurial effect on water permeability, by conducting control experiments with regard to cell physiology. The contribution of ion channels to $P_{os}$ was examined. The membrane permeability to ethanol was investigated in the absence and presence of mercury, since Steudle and Tyerman (1983) found that the reflection coefficient of ethanol indicated a large frictional interaction. Finally, the applicability of the two models mentioned above, to the data was examined.

### Materials and methods

**Plant material**

*Chara corallina* was cultured in artificial pond water (APW), consisting of 1 mol m$^{-3}$ NaCl, 0.1 mol m$^{-3}$ CaCl$_2$ and...
0.1 mol m\(^{-3}\) MgCl\(_2\), under a 12 h day/night regime of fluorescent light. \textit{C. corallina} internodes were isolated and placed into fresh APW overnight, in order to standardize their osmotic condition, prior to experimentation.

**Measurement of water relations parameters**

The pressure probe technique, developed by Hásken \textit{et al.} (1987), was adapted to determine the water relations parameters \(L_p, P_o\) and \(o_o\) of \textit{C. corallina} internodes. The cells were mounted into an open-ended tubular chamber, with a minimal internal diameter, in order to promote rapid exchange of extracellular solutions, thereby minimizing the effect of unstirred layers. Following the insertion of the pressure probe into the protruding end of individual cells, hydrostatic and osmotic pressure relaxation experiments were conducted, subsequent to the stabilisation of cellular turgor pressure (P).

Hydrostatic experiments consist of direct measurement of pressure relaxations upon induced turgor pressure perturbations, which may result in end- or exosmotic flow. This essentially eliminates the effect of unstirred layers on \(L_p\) determinations, as compared to transcellular osmosis. \(L_p\) is inversely related to the half-time of the pressure relaxations \((T_{1/2})\), by the following equation:

\[
T_{1/2} = \frac{\ln 2V}{L_p A (\epsilon + \pi_t)}
\]

where \(V\) is the total cell volume, \(A\) is the surface area of cell, \(\pi_t\) is the intracellular osmotic pressure (related to initial turgor pressure, \(P_o\)) and \(\epsilon\) is the volumetric elastic modulus of the cell wall (Steudle and Zimmermann, 1974). \(\epsilon\) relates to the concomitant changes in \(P\) and \(V\) (equation 4), which can be obtained from hydrostatic experiments.

\[
\epsilon = \frac{V \Delta P}{\Delta V}
\]

Osmotic experiments are based on perturbations of the extracellular osmotic pressure \(\pi_o\), resulting in monophasic or biphasic pressure relaxations due to non-permeating and permeating solutes, respectively.

Biphasic relaxations present essentially the same data as hydrostatic experiments, where \(L_p\) is found from the \(T_{1/2}\) taken to reach a new \(P\). Biphasic relaxations are comprised of an initial water phase, followed by a slower solute phase, where \(P_o\) is restored as the solute enters the cell, equilibrating \(\pi_t\) and \(\pi_o\). The membrane permeability to nonelectrolytes is expressed in terms of the permeability coefficient \(P_e\), which was determined from the half-time of the solute phase \((T_{1/2})\) of the biphasic relaxations:

\[
T_{1/2} = \frac{\ln 2V}{A P_e}
\]

The reflection coefficient was measured, taking account of \(\epsilon\) and \(P_e\), as per Steudle and Tyerman (1983). Consequently, the interaction of solutes with cell membranes, in terms of the reflection coefficient of solutes (\(\alpha\)), was analysed in relation to the frictional model (equation 1) and the composite membrane model (equation 2).

**Mercurial effects on \(L_p, \epsilon, P_e\) and \(\sigma\)**

Osmotic and hydrostatic experiments were performed before and after single treatments per cell with HgCl\(_2\). Recordings were taken at regular intervals over time, assuming membrane integrity, while \(P_o\) remained constant. Mercapto ethanol was applied, following a significant effect of HgCl\(_2\), in order to determine reversibility of the mercury effect. These experiments were repeated, using pCMPS as the mercurial agent, on separate cells.

The inhibition of \(L_p\) was expressed in terms of \% inhibition:

\[
\% \text{ inhibition} = \frac{L_{p0} - L_{p1}}{L_{p0}} \times 100
\]

where \(L_{p0}\) is the initial hydraulic conductivity in APW and \(L_{p1}\) is the subsequent hydraulic conductivity after saturation of the effect of HgCl\(_2\).

**Activation energy**

\(L_p\) values measured within a 5–25°C temperature range, were fitted to an Arrhenius plot, in order to determine \(E_a\). \(E_a\) was determined for cells bathed in APW and subsequent to treatment with 300 mmol m\(^{-3}\) HgCl\(_2\).

**Membrane potential measurements**

Whole cell membrane potential difference (PD) was measured using a single electrode (Findlay and Hope, 1976), which was inserted into the vacuole of a cell simultaneously with the pressure probe. The microelectrode was filled with 3 kmol m\(^{-3}\) KCl, where salt leakage is insignificant, with respect to the size of the cell (Blatt, 1988). For these experiments cells were mounted in a shallow dish-like chamber, which enabled access to the single electrode and the pressure probe. Membrane potential was measured upon exposure to HgCl\(_2\).

**Chara corallina** cells were induced into the K-state by irrigating the chamber with a depolarizing solution, consisting of 30 mol m\(^{-3}\) KCl, 0.1 mol m\(^{-3}\) CaCl\(_2\) and 0.4 mol m\(^{-3}\) MES, at pH = 5 (Beilby, 1985). Membrane resistance was monitored by injecting current pulses of 5.0 nA, at a frequency of 0.1 Hz to the cells, using a Neuroprobe amplifier, with an input impedance of \(10^{11}\) \(\Omega\) (Model 1600, A-M system, USA). A reduction in the membrane resistance when the cells were bathed in the depolarizing solution, was interpreted to be due to the opening of potassium channels. Membrane resistance was not monitored in all experiments.

The K\(^+\) channel blockers DOD (100 mmol m\(^{-3}\)) and tetra-ethylammonium chloride (TEA\(^+\), 10 mol m\(^{-3}\)) were applied after inducing the cells into the K-state. These blockers consistently increased membrane resistance by about 50%. The effect on \(L_p\) was recorded for the different electrophysiological states of the cells.

**Controls in relation to physiological effects of Hg\(^2+\) on cells**

The effect of HgCl\(_2\) on cytoplasmic streaming was determined, as an indicator for intracellular ATP concentration in \textit{C. corallina} cells (Reid and Walker, 1983). Respiration rates upon HgCl\(_2\) treatment were monitored with a Clarke oxygen electrode. Finally, \(L_p\) was determined after the exposure of cells to the metabolic inhibitors, CCCP (5 mmol m\(^{-3}\)) and azide (1 mol m\(^{-3}\)).

**Results**

The mean \(L_p\) in APW was 2.6 (±0.6, SEM, \(n=4\) cells) \(\times 10^{-6}\) m s\(^{-1}\) MPa\(^{-1}\). \(L_p\) was reduced maximally by approximately a factor of 10 following the application of HgCl\(_2\). The mean value of \(L_p\) after the inhibition by 300 mmol m\(^{-3}\) HgCl\(_2\) was 2.5 (±0.45, SEM, \(n=4\) cells) \(\times 10^{-7}\) m s\(^{-1}\) MPa\(^{-1}\). It was possible to reverse the
inhibitory effect with 1 mol m\(^{-3}\) mercaptoethanol, restoring \(L_p\) close to \(L_p\) prior to treatment (Fig. 1). The percentage inhibition of \(L_p\), in relation to the calculated mean \(L_p\) of cells bathed in APW, ranged from 46–92% inhibition subsequent to treatment with \(\text{HgCl}_2\) concentrations \(\geq 10\) mmol m\(^{-3}\) (Fig. 2). The time-courses of percentage inhibition were fitted to a single exponential, and implied a time-dependent dose response (Fig. 2), which may be associated with the location of the site of inhibition in the cell. No significant difference between endosmotic and exosmotic \(L_p\) was evident before \((t\)-test, \(P=0.88)\) or after \((t\)-test, \(P=0.73)\) \(\text{HgCl}_2\) treatment. The \(\epsilon\) and \(\pi\) were found to be unaffected throughout the treatments and prior to cell death (data not shown). Cell death was interpreted in terms of a constant loss in cell turgor pressure, which occurred after approximately 1 h of exposure to \(\text{HgCl}_2\), for the range of doses tested. 1 mmol m\(^{-3}\) and 5 mmol m\(^{-3}\) \(\text{HgCl}_2\) were not observed to have an effect on \(L_p\) prior to cell death (slope of linear regression of \(L_p\) against time not significantly different from zero) over approximately 1 h of treatment. This suggests that the lethal effect of \(\text{HgCl}_2\) on the cells is very dominant, and further implies the need to distinguish between a direct effect on \(L_p\) and an effect ascribed to other non-specific effects on cell physiology and membrane integrity.

The activation energy of water flow, induced by pressure relaxations, was measured upon the saturated effect of 300 mmol m\(^{-3}\) \(\text{HgCl}_2\), and was found to increase from a mean of 21.0 kJ mol\(^{-1}\) (±7.5, SEM, \(n=5\) cells) to 45.7 kJ mol\(^{-1}\) (±10.9, SEM, \(n=5\) cells). A paired t-test showed that the increase was significant \((P=0.0031)\).

Cell incubation with the mercurial compound \(\text{pCMPS}\) (analogous to \(\text{pCMBS}\), which was found to inhibit \(L_p\) of \(C.\ corallina\) internodes by Wayne and Tazawa (1990)), failed to show a significant effect on \(L_p\) (Runs test, \(P>0.05, n=14\) cells) in the experiments, even for 1 mmol m\(^{-3}\) \(\text{pCMPS}\), described as the saturating concentration used by Wayne and Tazawa (1990). A maximum 45 min incubation period with 1 mmol m\(^{-3}\) \(\text{pCMPS}\) resulted in cell death, without a preceding effect on \(L_p\). It was not possible to determine whether the effect on \(L_p\) was simply delayed with respect to the membrane permeability of \(\text{pCMPS}\), should the inhibitory binding site be intracellular.

The data presented so far are based on hydrostatic pressure relaxation experiments. Complementary osmotic experiments were undertaken, showing no significant difference between osmotic and hydrostatic determinations of the parameters examined, where the reflection coefficient of the non-permeating solutes mannitol and sorbitol, was unaffected by \(\text{HgCl}_2\). However, the reflection coefficient of the permeating nonelectrolyte, ethanol, was found to decrease from 0.28 (±0.05, SD, \(n=5\) cells), in many cases reaching negative values (Figs 3, 4a) This
was accompanied by an apparent constant solute permeability coefficient \( P_s = 2.6 \pm 0.07 \) SD, \( n = 5 \) cells) \( \times 10^{-6} \) ms\(^{-1}\), as determined from the solute phase of the biphasic pressure relaxations (Fig. 4b). This relates to a simultaneous decrease in \( L_p \), as illustrated in Fig. 5. Preliminary experiments were conducted with \( n \)-butanol and methanol. Their respective reflection coefficients were found to decline upon \( \text{HgCl}_2 \) treatment (data not shown), although it was not possible to take many measurements before cell death occurred.

Pressure relaxation \( T_{1/2} \) measurements for cells which were induced into the K-state, revealed no significant difference to \( T_{1/2} \) measured at the hyperpolarized resting \( PD \) of the cells (Table 1) which corresponds to no significant difference in \( L_p \), since \( \eta_1 \) and \( \epsilon \) do not change. Further, the effect of \( K^+ \) channel blockers, applied subsequently to inducing the cells into the K-state, were found to have no effect on \( T_{1/2} \) (Table 1). The mean \( T_{1/2} \) of all treatments presented in Table 1 were hence found to be not significantly different (repeated measures ANOVA, \( P = 0.14 \)). The addition of blockers did not result in a difference between endosmotic and exosmotic \( L_p \) (\( t \)-test, \( P > 0.05 \)).

Rapid depolarization of membrane \( PD \), to approximately -20 mV, was found upon the application of greater than 1 mmol \( m^{-3} \) \( \text{HgCl}_2 \) to the cells (Fig. 6a). 1 mmol \( m^{-3} \) \( \text{HgCl}_2 \) resulted in slower depolarization of the \( PD \). Figure 6b shows the effect of 10–100 mmol \( m^{-3} \) \( \text{HgCl}_2 \) on the respiration rate of \( C. \text{corallina} \) cells. The dramatic effect of \( \text{HgCl}_2 \) noted in Fig. 6b was found to be very rapid for the range of concentrations tested. The addition of \( \text{HgCl}_2 \) also caused rapid cessation of cytoplasmic streaming for concentrations greater than 300 mmol \( m^{-3} \) (data not shown).

Control experiments undertaken in order to elucidate the possible non-specific metabolic effects of \( \text{HgCl}_2 \) on \( L_p \) were done using the metabolic inhibitors CCCP and azide. These resulted in no significant effect on \( L_p \) following treatment for about 90 min as judged by the linear regressions on \( L_p \) versus time which yielded slopes not significantly different from zero (\( P > 0.05 \), \( n = 3 \) cells).
Discussion

The presence of water channels in cell membranes of *C. corallina* internodes is proposed on the basis of the high $L_p$ and low $E_a$ values measured in this study, and the effect of HgCl$_2$, which was found to inhibit the rate of water flow across the membranes and increase $E_a$. The time-dependent dose-response effect of HgCl$_2$ on $L_p$ (Fig. 1), may relate to an intracellular site, which supports the results of Tazawa et al. (1996), who, however, found that this was not due to an effect on the tonoplast. The fact that pCMPS was not found to have an effect, could be attributed to its lower membrane permeability (Naccache and Sha’fi, 1983; Whitembury et al., 1984). Previous studies where pCMBS was found to have an effect, applied the mercurial at relatively high concentrations and for extended incubation times (Verkman, 1992; Wayne and Tazawa, 1990).

No polar movement of water was observed prior to, or following the application of HgCl$_2$ to the cells. This is in contrast to some other reports which applied the transcellular osmosis technique (Wayne and Tazawa, 1990; Tazawa et al., 1996). This may well be due to a difference in technique, as no such polarity has been recorded for hydrostatic experiments using the pressure probe. The difference may be related to the effects of osmotic pressure on the water channels in *Chara* (Kiyosawa and Tazawa, 1972; Steudle and Tyerman, 1983).

Water transport may occur via a variety of membrane transport proteins, such as glucose transporters (Fischbarg et al., 1990). Large $K^+$ fluxes are often observed in relation to cellular turgor regulation events, such as turgor-driven stomatal aperture perturbations (Blatt, 1992). Further, Homble and Very (1992) have implicated tonoplast $K^+$ channels in facilitating the simultaneous flux of $K^+$ ions and water. Within this study, it was noted no significant effect or contribution of $K^+$ channels on the hydraulic conductivity of *C. corallina* membranes. Wayne and Tazawa (1990) have previously reported an effect of the $K^+$ channel inhibitor $C_9$ on endosmotic permeability.

The permeation of ethanol was examined before and after the inhibition of $L_p$ by HgCl$_2$. Notably, mercury did not have a significant effect on $P_t$ (Fig. 4b), whereas the reflection coefficient of ethanol declined in conjunction with a decrease in $L_p$ (Fig. 5). This can readily be described by the frictional model presented in equation 1, which assumes that the major pathway of water is via aqueous channels. If the major pathway of ethanol across the membrane is via the lipid bilayer, one would not expect $P_t$ to be affected significantly, when a large proportion of the mercury-sensitive water channels in the membrane are blocked. Further, the last term of equation 1 would be expected to remain constant because it is independent of the total number of open channels (but see below).

The low or even negative values of $a_e$ can hence be related back to equation 1, where the second term on the right side increases as $L_p$ decreases, resulting in a decreased value of $a_e$. The measured corresponding values of $a_e$ and $L_p$ were reasonably well fitted by equation 1, as presented in Fig. 5 (line a). Consequently, the combined frictional term in equation 1 was calculated to be 0.77, which is large and suggests a significant solute-solvent interaction within the aqueous pores.

Further, it is possible to obtain an independent estimate of $P_t$ from this fit (Tyerman and Steudle, 1982; Steudle and Tyerman, 1983). The fitted value of $P_t$ was found to
be $3.2 \times 10^{-6} \text{ m s}^{-1}$, which corresponds closely to the directly measured value of $2.6 \times 10^{-6} \text{ m s}^{-1}$. Hence, the frictional pore model does appear to be applicable to this system.

However, the assumptions implicit in equation 1 may not apply when most of the aqueous water channels are blocked. As the mercury sensitive channels are blocked, the relative contribution of water flow across the lipid bilayer or other mercury insensitive pathways, must be taken into account. Equation 7, from Dainty and Ginzburg (1963), represents the general case for a homogeneous membrane, where both water and the solute may move across the membrane via the lipid and/or aqueous channel pathways.

$$a_s = 1 - \frac{V_p P_s}{L_p R T} - \frac{K_s f_m}{f_m^* f_m} \left\{ \frac{(P_m *)_{hp} + \frac{L_p m}{L_p c}}{1 + \frac{L_p m}{L_p c}} \right\}$$

where $\phi_s$ is the volume fraction of the solute; $L_p m$ and $L_p c$ are the hydraulic conductivity of the lipid pathway and the aqueous pores, respectively. As water channels are blocked by $\text{HgCl}_2$, one expects $L_p c$ to decrease and hence the last term of equation 7 to decrease. Line b in Fig. 5 was obtained upon fitting the data to equation 7, where $L_p m$ is set equal to $L_p$ at the maximum 10-fold $\text{HgCl}_2$-inhibition, which represents an upper limit for this value, and $L_p c$ is the $\text{HgCl}_2$-inhibited component. This fit is contrary to the trend in the data (Fig. 5, line a). It is therefore predicted, on the basis of equation 7, that there must be an alternative, mercury-insensitive pathway, which promotes the large frictional interaction between the solute and water, assuming that the block of mercury-sensitive aqueous pores is complete. In this case, equation 7 can once again essentially be reduced to equation 1, thereby purely incorporating the frictional interactions within the mercury-insensitive pores. Line c of Fig. 5 was calculated on the basis that the membrane $L_p m$ was 10$^3$-fold lower than the total pore $L_p c$, in which case $\text{HgCl}_2$ only inhibited a small fraction of the pores. Line a and line c are relatively similar, indicating that equation 1 may be applied only if the pores responsible for the frictional interaction are not blocked substantially by $\text{HgCl}_2$.

Alternatively, the composite membrane model essentially describes pathways in terms of arrays, which consist of distinct water relations parameters (equation 2) (Henzler and Steudle, 1995). Here it is assumed that there are two distinct arrays, array $a$ and array $b$, representing selective water channels and the lipid bilayer respectively. If it is assumed that the lipid bilayer represents the mercury-insensitive pathway for ethanol, with a constant $\gamma L_p b$, then subsequent to $\text{HgCl}_2$-treatment, $a_s$ can be calculated from the following equation:

$$a_s = a_s - \gamma b \left( a_s - a_b \right) \frac{1}{L_p}$$

(Henzler and Steudle, 1995), which was closely fitted to the data of Fig. 5. The estimated value of $a_s$ was found to be 0.22, which is somewhat less than the value of 0.4, presented by Henzler and Steudle (1995). Both values are lower than the expected value of $\approx 1$, for highly selective water channels. The composite membrane model also appears to be applicable to our results, due to the close fit of equation 8 to the data of Fig. 5. This is not surprising since equation 8 is in a similar form to equation 1 with respect to $a_s$ and $L_p$. The composite membrane model, however, does not explicitly consider frictional forces within the pores, which may account for unexpectedly low estimates of $a_s$. Henzler and Steudle (1995) discuss a low $a_s$ in terms of a single-file mechanism in the pore.

Hence, both models fit this system equally well, although an independent estimate of $P_s$ can be obtained from the frictional model which cannot be done with the composite model. However, caution must be applied with respect to the complexity of cell membranes. Consistent with the apparently large frictional term, implying significant solvent drag forces, the possibility of other, less selective pathways must be taken into account.

The physiological effects of mercurial compounds must also be taken into consideration. It has been shown here that the depolarization of the membrane potential caused by $\text{HgCl}_2$ is probably not instrumental in causing a decrease in $L_p$ since membrane depolarization caused by other treatments did not affect $L_p$. Also the membranes did not appear to become leaky throughout the time that measurements of $a$ and $L_p$ were taken, in view of the constant $P_s$ and turgor values, and that the inhibition of $L_p$ was reversible with mercaptoethanol. Although the respiratory inhibitors azide and CCCP had no effect on $L_p$, the drastic effect on cyclosis, respiration and membrane potential by $\text{HgCl}_2$, must be considered. It is important to conduct a wide range of control experiments in order to estimate the possibility of secondary effects due to increased cation leakage among others (Kochian and Lucas, 1982; Lukacovic et al., 1984).

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