Onion Root Water Transport Sensitive to Water Channel and K⁺ Channel Inhibitors

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Transroot osmotic water flux (Jₐ) and radial hydraulic conductivity (Lₚₐ) in onion roots were greatly increased by three means; infiltration of roots by pressurization, repetition of osmosis and chilling at 5°C. Jₐ was strongly reduced by the water channel inhibitor HgCl₂ (91%) and the K⁺ channel inhibitor nonyltriethylammonium (C₉, 75%), which actually made the membrane potential of root cells less sensitive to K⁺. C₉ decreased the rate of turgor reduction induced by sorbitol solution to the same extent as HgCl₂. Thus, C₉ is assumed to decrease the hydraulic conductivity (Lₚ) of the plasma membrane by blocking water channels, although possible inhibition of the plasmodesmata of the root symplast by C₉ cannot be excluded. Onion roots transported water from the tip to the base in the absence of the osmotic gradient. This non-osmotic water flux (Jₑ) was equivalent to Jₐ induced by 0.029 M sorbitol. Jₑ increased when Jₐ was increased by repetition of osmosis and decreased when Jₐ was decreased by either HgCl₂ or by C₉. The correlation between Jₑ and Jₐ suggests that non-osmotic water transport occurs via the same pathways as those for osmotic water transport.

Key words: Hydraulic conductivity — K⁺ channel inhibitor — Membrane potential — Non-osmotic water transport — Onion roots — Water channel inhibitor.

Abbreviations: APW, artificial pond water; C₉, nonyltriethylammonium; Eₗ, membrane potential; Jₑ, transroot non-osmotic water flux; Jₐ, transroot osmotic water flux; Lₚ, cell hydraulic conductivity; Lₚₐ, transroot hydraulic conductivity; TEA, tetraethylammonium.

Introduction

Osmotic water permeability of barley roots (Lₚ) has been measured by the method of transroot osmosis (Tazawa et al. 1997). Namely, an isolated root is partitioned into two chambers, and water movement between the root tip and the base is induced by applying an osmotic gradient between the two parts of the root. Water transport or Lₚ is greatly increased by repetition of osmosis, and strongly decreased by HgCl₂ in both infiltrated and non-infiltrated roots. The third finding suggests that in barley roots water channels are involved in radial water transport from the external medium to the xylem vessels. Although transcellular, symplastic and/or apoplastic pathways are assumed for the radial water transport, it is most likely that combinations of these pathways are involved (Steudle and Peterson 1998, Taura et al. 1988). The strong reduction of Lₚ by HgCl₂ amounting to about 90% suggests that in the radial water transport, water should pass the plasma membrane at least once and that the contribution of the apoplast to the overall radial water flow may be minor. In order to know whether or not the characteristic of transroot osmosis found in barley roots (Tazawa et al. 1997) is generally applicable, we selected another monocot Allium cepa L. for study and compared the osmotic behavior of onion roots with that of barley roots. Both behaved similarly in that root infiltration and repetition of osmosis greatly increased water transport. Additionally, we found that water transport in onion roots was significantly increased by chilling treatment.

The non-osmotic water transport (Jₑ), which occurs from the tip to the base in the absence of the external osmotic gradient, is thought to be coupled with the transport of K⁺, because the main salts of the exudate from the cut end of maize roots are potassium salts (House and Findlay 1966, Collins and Reilly 1968, Anderson and Collins 1969, Dunlop and Bowling 1971, Miller 1985). In order to study the coupling between the non-osmotic water transport and K⁺ transport, we examined the effects of a K⁺ channel inhibitor, nonyltriethylammonium (C₉), on the transroot water movement. To clarify the contributions of the apoplastic and symplastic pathways to the non-osmotic water transport, we used HgCl₂ which is thought to decrease symplastic water transport strongly by inhibiting water channel activity (Tazawa et al. 1997).

Materials and Methods

Plant materials

Bulbs of onion (Allium cepa L.) purchased from the local market were placed in either a glass or polyethylene bottle filled with tap water in an air-conditioned room at 25°C under a light/dark regime of 15 h/9 h. After 7–10 d of culture, newly developed roots grew up to about 10 cm in length. Before use the roots were excised and stored for several h in artificial pond water (APW) containing 0.1 mM each of KCl, NaCl and CaCl₂. For infiltration of roots by pressurization, hydrostatic pressure amounting to about 3 bars was applied to a root placed in an injection syringe containing APW.

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Chilling treatment

Isolated roots incubated in APW were placed in a refrigerator with the temperature controlled at about 5°C. After overnight incubation the roots were brought back to room temperature of 20–24°C.

Measurement of transroot water movement

The transroot water movement was measured with a double-chamber volumeter (Tazawa et al. 1997). Briefly, the root (r) was partitioned into two parts through the partition wall with a groove into which the root was embedded with a small amount of lanolin. Normally the apical part about 40 mm long was placed in an open pool (A) and the basal part about 10 mm long was in a closed chamber (B) connected to a glass capillary (C) (Fig. 1). An air bubble (b) placed in the capillary served as the indicator of transroot water movement. The opening of the chamber B was sealed with a plexiglass block (s) using lanolin.

To induce transroot osmosis, APW in A was replaced with APW containing sorbitol. The volume of water transported from B to A was indicated by the distance of movement of the air bubble (b).

Fig. 1 Upper and side view of a double-chamber osmometer made of plexiglass for measuring transroot water movement. An isolated root (r) is embedded in the groove of the partition wall with lanolin wax so that the apical part (about 40 mm) is in open pool A and the basal part (about 10 mm) in closed chamber B. A sealing block (s) is fitted on the partition wall with lanolin to make the junction between the two compartments watertight. Pool A and chamber B are filled with APW. After filling chamber B with APW and introducing the air bubble (b) into the capillary (C), the hole (h) is closed with a plastic tap (t). Transroot osmosis is induced by replacing APW in A with APW containing sorbitol. The volume of water transported from B to A is indicated by the distance of movement of the air bubble (b).

Radial hydraulic conductivity of the root (Lpr) was calculated with the following formula:

\[ L_{pr} = -\frac{J_{os}}{\pi_{os}} \]  

(1)

where \( J_{os} \) and \( \pi_{os} \) represent the initial water flux at the start of osmosis and the osmotic pressure of the sorbitol solution, respectively. The water flux is the volume of water transported across the unit surface area of the root in the unit time and calculated by the following equation:

\[ J_{os} = \left(\frac{dv}{dt}\right)_{os} \frac{S_{a}}{S_{u}} \]  

(2)

where \( \left(\frac{dv}{dt}\right)_{os} \) and \( S_{a} \) are the initial rate of water flow and the surface area of the root in pool A, respectively.

In equation (2), we assumed that the transroot osmosis from B to A starts immediately after addition of a sorbitol solution. Actually, however, a small amount of water initially moved from A to B, namely in the direction opposite to the predicted one (Fig. 4). This minute water flow may be accounted for by the canal model developed by Taura et al. (1988) for radial water transport in roots. The model assumes two apoplast canal systems, an absorption canal existing in the peripheral region and an excretion canal in the stele. At the very beginning of application of the sorbitol solution, the sorbitol may act immediately on the external surface of the epidermal cells but not on the cell surface in the absorption canal. Water is lost from the epidermal cells, and there is a decrease in the turgor pressure of the symplast extending from the epidermis to the whole cortex (Fig. 2 in Taura et al. 1988). The turgor reduction increases the driving force for water flow from the canal to the symplast and results in a transient flow of water from the tip to the base of the root (cf. equation (2) in Taura et al. 1988). The flow ceases when sorbitol diffuses into the canal.

Isolated onion roots transported water from the apical part (A) to the basal part (B) even when no osmotic gradient existed between the two compartments containing APW. For convenience we call the exuding water movement the non-osmotic water transport. The non-osmotic water flux (\( J_{nos} \)) was calculated with the following equation:

\[ J_{nos} = \frac{\left(\frac{dv}{dt}\right)_{nos}}{S_{u}} \]  

(3)

where \( \left(\frac{dv}{dt}\right)_{nos} \) means the rate of exuding water flow.

Measurement of root turgidity

The turgidity of the root was measured by the turgor balance (Fig. 2, Tazawa 1957) as reported by Tazawa and Okazaki (1997). The essence of the method is to measure the deformation of the root on which a constant weight is loaded. Replacing APW with a sorbitol solution, the root tissue loses water, resulting in a decrease in the turgidity. The rate of decrease in the turgidity therefore represents the relative value of the hydraulic conductivity of the root cells.

Measurement of the potential difference between the apical and basal parts of the root

An isolated onion root was partitioned between two pools, the apical part in pool A and the basal part in pool B. The potential differ-
Onion root water transport

ence (P.D.) between the two pools (E_b – E_a) was measured with the external electrodes as shown in Fig. 3a. Ag-AgCl electrodes were connected to pools A and B through 3 M KCl and 2% agar containing 100 mM KCl. The P.D. was amplified with an amplifier and recorded with a pen-writing recorder (REC). First, both pools were filled with APW. Next, the medium in A was changed successively to APW supplemented with 1 mM, 10 mM and 100 mM KCl, while the ionic concentration in B was not changed. To eliminate the imbalance of the osmotic pressure caused by KCl, sorbitol was added to APW in B, i.e. 1.8, 18 and 180 mM corresponding to 1, 10 and 100 mM KCl, respectively. Since the ionic concentration in pool B was kept constant, changes in P.D. were considered to reflect changes in the electric potential of the apical part.

Measurement of membrane potential of root cells

The conventional glass microelectrode method was used to measure the membrane potential of root cells (Fig. 3b). A root segment between 15 and 30 mm from the apical tip was cut off and fixed at two loci between the fixed plexiglass plates p1 and p3 and the movable plates p2 and p4 with vaseline at the bottom. μ, glass microelectrode; ref, reference electrode connected to the earth.

Effect of repetition of osmosis and infiltration of roots on hydraulic conductivity

In barley roots, the rate of water transport induced by sorbitol was increased by repeating the osmosis and also by infiltration of the roots by pressurization (Tazawa et al. 1997). For onion roots, similar effects were observed. In Fig. 4 a non-infiltrated onion root was subjected to transroot osmosis which was induced by 0.2 M sorbitol. The rate of osmosis increased remarkably with repetition of osmosis. This agrees with the results obtained with barley roots.

In barley, infiltration of the roots by pressurization greatly increased Lp_r (Tazawa et al. 1997). The same was found for onion, with Lp_r significantly increasing from 0.014 ± 0.011 pm s^{-1} Pa^{-1} (n = 9) to 0.056 ± 0.042 pm s^{-1} Pa^{-1} (n = 10) (Tazawa et al. 1997). Thus, the osmotic behavior of onion roots is the same as that of barley roots.

Effect of low temperature treatment on hydraulic conductivity

Treatment of roots with low temperature (5°C) increased Lp_r. Fig. 5 shows that the rate of osmosis was increased about three times by treatment of the root with low temperature. Lp_r of the roots stored at 5°C (0.139 ± 0.114 pm s^{-1} Pa^{-1}, n = 4) was significantly higher than that of the roots stored at room temperature (0.015 ± 0.011 pm s^{-1} Pa^{-1}, n = 8).
Effect of infiltration and low temperature treatment on turgidity change

To measure the change in the turgidity of the root during transroot osmosis with a turgor-balance (Fig. 2), the non-infiltrated root was partitioned into two pools, the apical part in pool A and the basal part in pool B as illustrated in Fig. 1. A weight was loaded onto the apical part in A. When 0.2 M sorbitol was applied to the apical part of the root, transroot osmosis was induced. At the same time, the root started to deform. The deformation proceeded almost linearly reaching 45 μm after 300 s (curve 1 in Fig. 6). On infiltration of this root, the rate of deformation increased (curve 2). Overnight treatment of the same root with low temperature further increased the rate of deformation (curve 3). The results indicate that both infiltration and chilling enhance the decrease of root turgidity, indicating that the osmotic efflux of water from root cells is accelerated by infiltration as well as by chilling.

Effect of nonyltriethylammonium (C₉) on J_{non}, J_{os} and Lp

Onion roots transported water from the tip to the base in the absence of the osmotic gradient between the apical and basal portions. J_{non} is thought to be induced by exudation of solutes from the stelar parenchyma cells into the xylem vessels via the apoplast canals (Katou et al. 1987, Taura et al. 1988). Since the main salts in the xylem sap are K⁺-salts (House and Findlay 1966, Collins and Reilly 1968, Anderson and Collins 1969, Dunlop and Bowling 1971, Miller 1985, Pate 1975), transport of K⁺ from the external medium to the xylem vessels may be crucial for the non-osmotic flow. Nonyltriethylammonium (Br salt) known as a potent K⁺ channel inhibitor (Armstrong and Hille 1972, Tazawa and Shimmen 1980) was applied to the apical part of the root to reduce the K⁺ permeability of the plasma membrane. Application of 1 mM C₉ gradually decreased non-osmotic water transport (Fig. 7). After 30 min, the rate of water transport was decreased down to 17%
Onion root water transport

of the rate before \( C_9 \) treatment.

To obtain Fig. 8, both \( J_{nos} \) and \( J_{os} \) were measured successively in an infiltrated root. First, \( J_{nos} \) was measured, and then transroot osmosis was induced with 0.1 M sorbitol to get \( J_{os} \). Measurements at point 4 were done after treatment of the root with 1 mM \( C_9 \) for 30 min.

Comparing \( J_{nos} \) with \( J_{os} \) obtained under a 0.1 M osmotic gradient showed the driving force of the exuding water transport to be equivalent to about 0.029 M sorbitol. This value is comparable to the osmotic pressure of the root exudate of maize (0.042 Osm, House and Findlay 1966; 0.062 Osm, Miller 1985), Lupinus (0.017 Osm, Pate 1975) and paprika pepper (0.054 Osm, Carvajal et al. 1999).

**Effect of \( HgCl_2 \) on \( J_{os} \) and \( J_{nos} \)**

Mercuric chloride is known as a potent inhibitor of water channels in both plant and animal cells (Chrispeels and Agre 1994, Maurel 1997). To observe its effects, both \( J_{os} \) and \( J_{nos} \) were measured first in the absence and then in the presence of 100 \( \mu \)M \( HgCl_2 \). As shown in Table 2, \( HgCl_2 \) led to a decrease in both fluxes by more than 80%. This supported the intimate correlation between \( J_{os} \) and \( J_{nos} \) shown in Fig. 8.

**Table 1** Effects of \( C_9 \) on \( J_{nos} \), \( J_{os} \) and \( Lp_{r} \) in infiltrated onion roots

<table>
<thead>
<tr>
<th>( J_{nos} ) (nm s(^{-1}))</th>
<th>( J_{os} ) (nm s(^{-1}))</th>
<th>( Lp_{r} ) (pm s(^{-1}) Pa(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( C_9 )</td>
<td>Control ( C_9 )</td>
<td>Control ( C_9 )</td>
</tr>
<tr>
<td>Mean 14.4</td>
<td>2.3</td>
<td>49.6</td>
</tr>
<tr>
<td>( \pm SD ) 6.6</td>
<td>1.6</td>
<td>41.1</td>
</tr>
<tr>
<td>( n ) 8</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

\( J_{os} \) is the water flux induced by the osmotic gradient of 0.1 M. Roots were treated with \( C_9 \) for 30–50 min.

**Table 2** Effects of \( HgCl_2 \) on \( J_{nos} \), \( J_{os} \) and \( Lp_{r} \) in non-infiltrated onion roots

<table>
<thead>
<tr>
<th>( J_{nos} ) (nm s(^{-1}))</th>
<th>( J_{os} ) (nm s(^{-1}))</th>
<th>( Lp_{r} ) (pm s(^{-1}) Pa(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( HgCl_2 )</td>
<td>Control ( HgCl_2 )</td>
<td>Control ( HgCl_2 )</td>
</tr>
<tr>
<td>Mean 7.5</td>
<td>1.2</td>
<td>24</td>
</tr>
<tr>
<td>( \pm SD ) 1.6</td>
<td>0.1</td>
<td>8</td>
</tr>
<tr>
<td>( n ) 3</td>
<td>1</td>
<td>0.028</td>
</tr>
</tbody>
</table>

The number of roots used was three. Roots were treated with 100 \( \mu \)M \( HgCl_2 \) for about 40 min. \( J_{os} \) is the water flux induced by 0.1 M sorbitol osmotic gradient.

**Table 3** Effects of \( C_9 \) and \( HgCl_2 \) on the rate of deformation of infiltrated onion root segments induced by sorbitol solution

<table>
<thead>
<tr>
<th>Rate of deformation (( \mu )m s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( C_9 )</td>
</tr>
<tr>
<td>Mean 0.64</td>
</tr>
<tr>
<td>( \pm SD ) 0.59</td>
</tr>
<tr>
<td>( n ) 7</td>
</tr>
</tbody>
</table>

The concentration of sorbitol solution was 0.3 M for \( C_9 \) experiment and 0.2 M for \( HgCl_2 \) experiment. Before measurement roots were treated with \( C_9 \) for about 100 min or with \( HgCl_2 \) for about 35 min.

**Effect of \( C_9 \) and \( HgCl_2 \) on turgidity change induced by sorbitol**

Turgidity change induced by sorbitol was measured under the transroot osmosis conditions. When a sorbitol solution is supplied to the apical part of the root (A in Fig. 1), the sorbitol may diffuse into the peripheral apoplastic space including the epidermis and hypodermis (Taura et al. 1988) and draw water from the cells forming a symplast (Robards and Clarkson 1976). When \( C_9 \) decreases the osmotic water permeability of root cells, it may decelerate the rate of deformation of the root induced by the sorbitol solution. The deformation rate which had been 0.32 ± 0.12 \( \mu \)m s\(^{-1}\) \((n = 4)\) was decreased to 0.12 ± 0.02 \( \mu \)m s\(^{-1}\) by treatment of the roots with \( C_9 \).

In order to compare the effect of \( C_9 \) with that of \( HgCl_2 \), experiments were done on root segments obtained by cutting roots at 15, 30 and 45 mm from the tip. Segments of either 15–30 mm or 30–45 mm were used. Sorbitol may diffuse not only...
Onion root water transport 33

into the cortical apoplast but also to the stelar apoplast of the segment. Table 3 shows that the rate of deformation was decreased to 34 and 23% of the control by C₉ and HgCl₂, respectively. Thus C₉ reduced Lₚ of root cells to nearly the same extent as HgCl₂, suggesting that C₉ influenced the water channel activity as HgCl₂.

Effect of C₉ and HgCl₂ on sensitivity of membrane potential (Eₘ) to \([K^+]_o\]

If C₉ decreases K⁺ channels, Eₘ may be less sensitive to K⁺ in the presence of C₉ than in its absence. Fig. 9 shows that the P.D. between the apical and basal parts was affected by changing the K⁺ concentration of the medium bathing the apical part. The sensitivity of the P.D. to K⁺ was significantly decreased by treatment of roots with 1 mM C₉.

Next, Eₘ of cortical cells was measured to see the direct effect of C₉ on the sensitivity of Eₘ to K⁺. Fig. 10 shows that Eₘ became less sensitive to K⁺ after treatment of the roots with C₉. Depolarization of Eₘ with C₉ was also observed in Nitellopsis cells (Wayne and Tazawa 1990) and with TEA in squid giant axon (Armstrong and Binstock 1965). The above results show that C₉ may decrease the K⁺ permeability of the plasma membrane in onion root cells as in characean cells.

If a causal relationship exists between the reduction in Lₚ and the reduction in the K⁺ permeability, then HgCl₂ might also have an inhibitory effect on the K⁺ permeability as C₉ does. However, when roots were treated with 100 μM HgCl₂ for more than 30 min and the sensitivity of the P.D. to \([K^+]_o\) was examined, unlike C₉ (Fig. 9), HgCl₂ was found not to affect the K⁺-sensitivity of the plasma membrane (Fig. 11).
Discussion

Enhancement of Lp, by infiltration of roots and repetition of osmosis

The radial hydraulic conductivity (Lp,) in onion roots was increased by both infiltration of roots and repetition of trans-root osmosis (Fig. 4) as in barley roots. The increase was attributed to an increase in the area of the plasma membrane available for water transport (Tazawa et al. 1997). Before infiltration, the apoplastic space containing gas may not be available to water transport. After infiltration, the gas is replaced with aqueous solution so that the increased wet surface of the plasma membrane can be used for transport of water and solutes. Roots before infiltration look opaque due to light scattering but become transparent after infiltration. Roots after repetition of osmosis also appear transparent.

Passioura (1988) discusses the possibility of the artefactual conductus for the longitudinal transport of water being formed in the cortical apoplast when roots are infiltrated by pressurization. However, infiltration increases Lp, significantly in both barley (Tazawa et al. 1997) and onion roots. If the increase in Lp, is due to the formation of apoplastic longitudinal pathways as suggested by Passioura (1988), the increment of Lp, should be insensitive to HgCl2. However, contrary to this, in barley roots, the increased Lp, after infiltration is more sensitive to HgCl2 than Lp, before infiltration (Tazawa et al. 1997).

Chilling

Increase in Lp, by chilling treatment may be explained in terms of infiltration of the apoplastic space. Roots after overnight chilling looked transparent. Freshly isolated roots placed in a beaker filled with APW floated on the water. However, the roots after chilling were either submerged completely at the bottom of the beaker or suspended in water in an upright position with the tip directed downward. The infiltration of roots by chilling may be due to an increase in the solubility of gases and also to a decrease of gas production at low temperature.

Comparison with the pressure method

Melchior and Steudle (1993) measured water transport of onion roots using a root pressure probe in which pressure was applied to the xylem vessel to induce radial water flow. The value of Lp, was of the same order of magnitude (about 0.14 pm s⁻¹ Pa⁻¹) as that obtained in the present study using the transroot osmosis method (0.1–0.2 pm s⁻¹ Pa⁻¹). Quite recently, Barrowclough et al. (2000) measured Lp, of different zones of an intact onion root and found that Lp, for the youngest zone (30–40 mm from the tip) was 0.15 pm s⁻¹ Pa⁻¹ which was significantly smaller than those (0.24 and 0.28 pm s⁻¹ Pa⁻¹) of the middle (55–65 mm) and the oldest zone (100–110 mm). The value for the youngest zone agrees well with our data obtained from the apical 40 mm portion of an isolated root (Table 1 and 2).

Nature of Jnos

The water transport occurring even in the absence of the external osmotic gradient may be identical with the exudation of the xylem sap from detached roots and is assumed to be driven by active transport of solutes into the xylem (Passioura 1988) or into apoplast canals surrounding the symplast of stelar parenchyma cells (Katou et al. 1987, Taura et al. 1988). Root cells form symplastic structures connected with plasmodesmata which run from the root epidermis, through the cortex, across the endodermis and the pericycle to the xylem parenchyma cells adjacent to the vessel elements (Robards and Clarkson 1976).

Ginsburg and Ginzburg (1970, 1971) prepared a maize root from which the central stelar portion was removed. The “sleeve” which is composed of epidermis, cortex and endodermis behaved as though it was a single cell having a semipermeable membrane with a reflection coefficient of 0.98–0.99 for both sucrose and NaCl. The sleeve transported water even in the absence of the osmotic gradient between the inner and outer compartments (Ginsburg and Ginzburg 1970). The non-osmotic flux amounted to about 10 nm s⁻¹ which is about the same as Jnos of onion roots (Table 1 and 2). In maize roots, both Jos and Jnos were decreased by about 50% by 1 mM KCN or by 10 μM DNP (Ginsburg and Ginzburg 1971). Also in wheat root cells, Lp, was strongly reduced by hypoxia and NaN3 (Zhang and Tyerman 1991) to the same extent as by HgCl2 (Zhang and Tyerman 1999). Water channels which are sensitive to HgCl2 may also be closed by metabolic inhibition. Thus HgCl2 may affect water channels not directly but indirectly by inhibiting cell metabolism (Tyerman et al. 1999). An alternative is that the conductance through the plasmodesmata may be sensitive to metabolic inhibitors as discussed later.

In Chara cells, however, CCCP and NaN3 had no effect on Lp (Schütz and Tyerman 1997). The cytosolic ATP level had also no effect on Lp (Wayne et al. 1994). The mechanism of water transport in roots is more complex than that in a single cell of Chara, since water transport in roots is coupled with uptake of nutrients such as NO3⁻, H2PO4⁻ and SO4²⁻ (Clarkson et al. 2000). Membrane transport of these anions is energy-dependent or coupled with the activity of the plasma membrane H⁺-ATPase (pp. 328–329 in Lütte and Higinbotham 1979, Goldstein and Hunziker 1985, Sakano et al. 1992).

The non-osmotic water flow, namely exudation from the xylem, may be counter-balanced by the osmotic water flow induced by a sorbitol solution of about 0.03 M which is applied to the apical end (Tables 1 and 2). In maize roots, in order to stop exudation from the root sleeve, the osmotic potential of the external solution should be 0.05–0.2 MPa lower than that of the exudate (Ginsburg and Ginzburg 1971). This gradient corresponds to 0.019–0.077 M sorbitol equivalent. Both Jnos and Jos were increased simultaneously by repetition of osmosis (Fig. 8), suggesting that non-osmotic water transport uses the same pathways as the osmotic water transport.
K⁺ channel inhibitor versus water channel inhibitor

K⁺ channel blocker C₉ (1 mM) decreased both Jₙos and Jₙs in onion roots by 84% and 75%, respectively (Table 1). It also decreased transcellular hydraulic conductance of *Nitellopsis* cells by 25% (Wayne and Tazawa 1990). In onion roots, another K⁺ channel blocker, tetraethylammonium (TEA, 10 mM), was less effective (40% reduction in Lp) than C₉. In tonoplast-removed cells of Chara, the light-induced activation of K⁺ channels was decreased by C₉ more effectively than by TEA (Tazawa and Shimmen 1980). In contrast to onion roots, TEA did not affect Lp of wheat root cells (Zhang and Tyerman 1999) and that of *Chara corallina* cells (Schütz and Tyerman 1997). We also confirmed the ineffectiveness of C₉ on Lp in *Chara corallina* (data not shown).

The reduction in Lp, by C₉ in onion roots (75% reduction, Table 1) was slightly less than that induced by HgCl₂ (92%, Table 2). Generally Lp of plant cells is very sensitive to HgCl₂ (Henzler and Steudle 1995, Tazawa et al. 1996, Tazawa et al. 1997, Schütz and Tyerman 1997, Carvajal et al. 1999, Zhang and Tyerman 1999, Barrowclough et al. 2000), which may be attributed to the sensitivity of the plasma membrane water channels to this drug (Kammerloher et al. 1994, Kaldenhoff et al. 1998). However, Lp of plasma membrane vesicles prepared from wheat roots was insensitive to HgCl₂ (Niemietz and Tyerman 1997), though Lp of intact root cells was sensitive to the same drug (Zhang and Tyerman 1999). Plasma membrane vesicles prepared from tobacco suspension-cultured cells were also insensitive to HgCl₂ (Maurel et al. 1997). These findings may be accounted for by assuming that the major part of the radial water transport in roots occurs not only across the plasma membrane but also through plasmodesmata which may be disrupted or closed during preparation of membrane vesicles (Niemietz and Tyerman 1997). Thus plasma membranes and plasmodesmata are assumed to represent two major pathways for symplastic water transport in roots. The reduction in Lp, is then interpreted either by inhibition of water channels of the plasma membrane of the root symplasts or inhibition of the plasmodesmata which is essential for the symplastic water transport. Then the question arises of whether or not C₉ decreases the radial water transport by affecting water channels or by affecting the plasmodesmata. Since the reduction of Jₙs by C₉ (75% reduction, Table 1) is slightly smaller than that of Jₙos by HgCl₂ (92% reduction, Table 2), C₉ is assumed to affect Lp of the plasma membrane via inhibition of water channels, although the possibility of inhibition of plasmodesmata by both C₉ and HgCl₂ cannot be excluded.

Nature of reduction of Lp by C₉

Why C₉ decreases Lp is not known at present. Recent data suggest that Lp of plant cells is affected not only by water channel blockers but also by metabolic inhibitors and nutrient deficiency. Thus Lp was reduced by hypoxia or NaNO₃ (Zhang and Tyerman 1991) to a level that is comparable to Lp decreased by HgCl₂ (Zhang and Tyerman 1999). Since Lp after hypoxia treatment was not sensitive to HgCl₂, the cell metabolism is assumed to have an effect on the water channel activity of the plasma membrane (Zhang and Tyerman 1999). Wheat roots cultured under starvation of either nitrogen or phosphorus showed a remarkably lower hydraulic conductivity compared with roots supplied with complete nutrient (Carvajal et al. 1996). Clarkson et al. (2000) have thoroughly discussed the effects of deficiency of nutrients such as NO₃⁻, H₂PO₄⁻ or SO₄²⁻ on the root Lp and Lp of root cells. They assume that a signal, probably reduction of nutrient fluxes in roots, may be sensed by plants, although the signal transduction pathway resulting in a decrease in root hydraulic conductivity is unknown. Nutrients which have been reported to affect Lp are anions. The reduction in Lp, by a K⁺ channel inhibitor may suggest that fluxes of K⁺, which is one of the essential nutrients, are linked with the activity of water channels in the plasma membrane.

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References


Onion root water transport


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