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ORIGINAL ARTICLE

Age dependence of the hydraulic resistances of the plasma membrane and the tonoplast (vacuolar membrane) in cells of *Chara corallina*

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Abstract
Hydraulic resistances (reciprocals of hydraulic conductivities) of the cell (Lp^{-1}), the cell wall (Lp_w^{-1}), the membrane (Lp_m^{-1}), the plasma membrane (Lp_{pm}^{-1}), and the tonoplast (Lp_{tp}^{-1}) were determined in individual internodal cells of *Chara corallina* and their dependence on the cell age was studied. The thickness of the cell wall (*d*) was adopted as an index of the cell age, since the cell wall of spring-grown young cells (sg-cells) was found to be significantly thinner than that of winter-spent old cells (ws-cells). Both Lp_w^{-1} and Lp_m^{-1} were found to increase with cell age. Since Lp_m^{-1} is the sum of Lp_{pm}^{-1} and Lp_{tp}^{-1}, their dependence on the wall thickness was studied. It was found that both Lp_{pm}^{-1} and Lp_{tp}^{-1} increase with cell age using *d* as a proxy and that the former is distinctly higher than the latter. The ratio $Lp_{pm}^{-1}/Lp_{tp}^{-1}$ amounts to 30 for 5 μm of *d*, indicating that the tonoplast is a negligible barrier to osmotic water flow. The ratio decreases with the increase in *d* and amounts to 5.0 for 11 μm of *d*, showing that the tonoplast ages faster than the plasma membrane. The physiological meaning of the age dependence of hydraulic resistance of the tonoplast was discussed in terms of the role of the vacuole in the osmoregulation of the cytoplasm.

Keywords Cell age · Cell wall · *Chara corallina* · Hydraulic resistance · Plasma membrane · Tonoplast

Introduction

In plant cells, the barrier components resisting osmotic water flow are the cell wall, the plasma membrane, and the tonoplast. The problem is to know the contribution of each barrier component to the whole resistance. Kamiya and Tazawa (1956) measured the hydraulic conductivity of an internodal cell of *Nitella flexilis* using the method of transcellular osmosis developed by Osterhout (1949). The hydraulic resistance (Lp^{-1}) of the cell, defined as the reciprocal of the hydraulic conductivity (Lp), is the sum of the hydraulic resistance of the cell wall (Lp_w^{-1}) and that of the membrane (Lp_m^{-1}) as shown in the Eq. (1).

$$Lp^{-1} = Lp_w^{-1} + Lp_m^{-1} \quad (1)$$

Lp_m^{-1} is the sum of the hydraulic resistance of the plasma membrane (Lp_{pm}^{-1}) and that of the tonoplast (Lp_{tp}^{-1}) as shown in the Eq. (2).

$$Lp_m^{-1} = Lp_{pm}^{-1} + Lp_{tp}^{-1} \quad (2)$$

To obtain Lp_w^{-1}, Kamiya et al. (1962) prepared the cell wall tube from an internodal cell of *Nitella flexilis* and determined Lp_w^{-1} by measuring the water flux that was induced by applying hydrostatic pressure to the inside of the cell wall tube.

Kiyosawa and Tazawa (1977) tried to determine the hydraulic conductivity of the tonoplast in cells of *Chara corallina*. They first measured Lp of an internodal cell. Then, the tonoplast of the cell was removed by perfusing the vacuole with a medium containing EGTA, a Ca^{2+}-chelating agent. The Lp of the tonoplast-free cell (^{tpf}Lp) was measured and compared with the Lp of the normal cell. Since no

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significant difference between Lp and ${}^{tpf}Lp$, was found, Kiyosawa and Tazawa (1977) concluded that the tonoplast is so permeable to water that its presence does not affect the cell water permeability (Lp). Consequently, the hydraulic resistance of the membrane (Lp_m^{-1}) was assumed to be almost equal to the hydraulic resistance of the plasma membrane (Lp_{pm}^{-1}). Namely,

$$Lp_m^{-1} \simeq Lp_{pm}^{-1} \quad (3)$$

A greater water permeability of the tonoplast than that of the plasma membrane symbolized by the relationship of $Lp_{tp} \gg Lp_{pm}$ was also found in higher plant cells. Url (1971) applied the plasmometry method to the protoplast and the vacuole of epidermal cells of the onion bulb scale and found that the Lp of the tonoplast is about 100 times higher than that of the plasma membrane. Taking advantage of the stopped-flow light-scattering method for the measurement of osmotic shrinking of membrane vesicles derived from the plasma membrane and the tonoplast from tobacco cultured cells, Maurel et al. (1997) found that the osmotic water permeability (P_f) of the tonoplast (690 μm s^{-1}) is about 100 times greater than that of the plasma membrane (ca.7 μm s^{-1}). Using wheat root cells, Niemietz and Tyerman (1997) prepared the plasma membrane fraction and the endomembrane fraction including tonoplast vesicles and measured the P_f of each fraction by using the light-scattering method. Also, in this material, P_f of the endomembrane fraction (86 μm s^{-1}) is about 7 times higher than that of the plasma membrane fraction (12.5 μm s^{-1}).

Morillon and Lassalles (1999) measured the P_f of the vacuoles isolated from various organs (leaf, hypocotyl, and root) of onion, rape, petunia, and red beet. Values of the P_f of the vacuoles were in the range of 200–1000 μm s^{-1} (Lp: 1.5–7.4 pm s^{-1} Pa^{-1}). These values are significantly larger than the P_f values of the protoplast ranging from 2.5 to 370 μm s^{-1} (Maurel et al. 2002).

Ohshima et al. (2001) prepared vesicles of the plasma membrane and tonoplast from radish root cells and from leaves of a CAM plant and determined their P_f values by measuring the osmotic volume changes. While the P_f of the plasma membrane could be measured as 13 and 2.8 μm s^{-1} for radish and the CAM plant, respectively, the P_f of the tonoplast could not be quantified because the volume change of the tonoplast vesicles was too rapid.

Differing from the results cited above, Murai-Hatano and Kuwagata (2007) reported that the plasma membrane is as permeable to water as the tonoplast. They found that the protoplast prepared from radish root was as permeable to water as the vacuole that was derived from the same protoplast.

One of the objects of the present study was to reexamine the validity of Eq. (3) used by Kiyosawa and Tazawa (1977) in their study of *Chara* cells. To achieve the goal, both Lp_{pm} and Lp_{tp} were determined. The result showed that Eq. (3) is valid in young cells having a thin cell wall. The second object was to see whether or not the hydraulic resistance of the membrane is related to the cell age. It was found that both Lp_{pm}^{-1} and Lp_{tp}^{-1} are dependent on the thickness of the cell wall which is assumed to be an index of the cell age: Lp_{tp}^{-1} is more sensitive to aging than Lp_{pm}^{-1}.

Material and methods

Plant material

As the experimental material, internodal cells of *Chara corallina* were used. The plant was cultured outdoors in buckets containing tap water. To enhance growth of the alga, small amounts of dried oak leaves were added into the culture buckets. To prevent freezing in winter, each bucket was covered with a plate of polyacrylate resin on which a sheet of polyethylene was placed.

Internodal cells were isolated from neighboring internodal cells and stored in tap water. Before each experiment, cells were transferred to deionized water which was made by passing the tap water through a Cartridge Deionizer (Type G-10C, Organo, Tokyo). The electric conductivity of the water was less than 10^{-4} S m^{-1}.

Test solutions

Sorbitol solutions were used to induce transcellular osmosis. The osmotic pressures of experimental solutions were measured with the WESCOR vapor pressure osmometer (MODEL 5520, *WESCOR* Inc., UT, USA). The vacuolar perfusion medium (EGTA-medium) used to remove the tonoplast contained 5 mM EGTA, 6 mM $MgCl_2$, 30 mM PIPES, 67 mM KOH, 1 mM ATP, and 150 mM sorbitol (Shimmen and Tazawa 1982). The osmolality of the EGTA medium was 250 mOsm, which is about isotonic to the osmotic pressure of *Chara* cells. The osmotic pressure of the cell was measured by the turgor balance (Tazawa 1957).

Determination of the hydraulic resistance of the cell (Lp^{-1}) by transcellular osmosis

The hydraulic resistance of an internodal cell (Lp^{-1}) was measured by the method of transcellular osmosis (Osterhout 1949; Kamiya and Tazawa 1956; Dainty and Ginzburg 1964; Kiyosawa and Tazawa 1972). The measuring apparatus consists of two chambers, A and B (Fig. 1). An internodal cell is partitioned symmetrically between two chambers A and B which are filled with deionized water (water). The central part of the cell is embedded into the groove with lanolin paste so

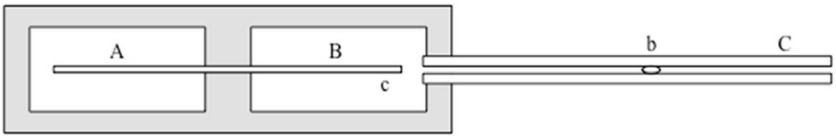

Fig. 1 Double chamber osmometer for measuring transcellular osmosis. An internodal cell (c) is partitioned into two chambers A and B across the partition wall. The volume of water transported by the transcellular osmosis is indicated by the movement of the air bubble (b) in the capillary C. The movement is traced using the ocular micrometer of the traveling microscope

that water movement between both chambers is possible only through the cell.

To induce transcellular osmosis, water in A was replaced with a 0.1 M sorbitol solution ($\pi_o = 2.51 \times 10^5$ Pa). This concentration was chosen, since higher concentrations inhibit *L*p (Dainty and Ginzburg 1964; Kiyosawa and Tazawa 1972; Ye et al. 2004). The volume of water transported from B to A through the cell was indicated by the shift of the air bubble (b) in the capillary (C). The initial rate of water flux (J_v) is proportional to the osmotic pressure of the external solution (π_o) as shown by Eq. (4).

$$J_v = K\pi_o \tag{4}$$

where *K* is the transcellular osmosis constant. To get J_v, the volume of water transported in the initial 60 s (*v*) was measured. *K* was calculated by the following equation derived from the kinetic analysis of transcellular osmosis (Kamiya and Tazawa 1956):

$$K = -1.84 \times 10^{-8}\ V \log\left(5\text{–}4e^{2V/v}\right) \tag{5}$$

where *V* is the volume of the cell without the cell part at the partition wall. *V* is in m^3 and *K* is in $m^3\ s^{-1}\ Pa^{-1}$.

When the cell is partitioned symmetrically, the whole resistance for the transcellular osmosis *R*, which is the reciprocal of *K* (K^{-1}), is the sum of the resistances of the endosmotic and the exosmotic cell halves. Since the resistance for the osmosis is proportional to the hydraulic resistance (Lp^{-1}) and inversely proportional to the surface area of the cell, the resistance of the cell half (*R*/2) is expressed as $R/2 = K^{-1}/2 = Lp^{-1}/(A/2)$. Then, *L*p is expressed by Eq. (6):

$$Lp = 4K/A \tag{6}$$

where *A* is the surface area of the cell without the cell part at the partition wall. From Eqs. (5) and (6), we get:

$$\begin{aligned} Lp &= -7.36(V/A)\log\left(5\text{–}4e^{2V/v}\right) \times 10^{-11}\ m\ s^{-1}\ Pa^{-1} \\ &= -3.68r\log\left(5\text{–}4e^{2V/v}\right) \times 10^{-11}\ m\ s^{-1}\ Pa^{-1} \end{aligned} \tag{7}$$

where *r* is the radius of the cell.

Measurement of the hydraulic resistance of the cell wall (Lp_w^{-1})

The Lp_w^{-1} was determined in the cell wall tube prepared from the cell by using the apparatus shown in Fig. 2 which was essentially the same as that used by Kamiya et al. (1962). It consists of three parts, a glass syringe (Sy) with a driving screw (Sc), a pressure gauge (G), and a measuring pipette (P).

A right-angled glass capillary (C) was prepared. One end of the capillary was inserted into the pipette, and the junction was fixed with sticky wax. The cell wall tube (cw) was prepared as follows: an internodal cell was bathed in a slightly hypertonic sorbitol solution (0.3 M) to abolish the turgor. One end of the cell was cut with scissors. The cell content was squeezed out with a finger. The opened end of the cell wall tube (cw) was slipped onto the glass capillary (C), and the contact area was cemented with sticky wax to keep the area waterproof. The outflow of water through the cell wall tube was induced by applying hydrostatic pressure inside the cell wall tube. The volume of water transported across the cell wall tube (cw) was indicated by the shift of the air bubble (b) in the pipette (P). The shift was followed with the eyepiece micrometer of the traveling microscope (TM PRM2, PIKA SEIKO Ltd., Tokyo, Japan). The time (*t*) to cover 100 units of the micrometer which amounts to the volume of water flowing through the cell wall tube (v_w: 2.84×10^{-9} m^3) was measured with a stopwatch. The pressure was measured with the pressure gauge (G; pressure range −0.1~0.4 MPa, Type AA10, Nagano Keiki, Tokyo, Japan). Lp_w was calculated by Eq. (8):

$$Lp_w = v_w/t\ P_w S_w \tag{8}$$

where P_w and S_w are the hydrostatic pressure (5×10^4 Pa) applied to the inside of the cell wall tube and the surface area of the cell wall tube, respectively. The thickness of the cell wall was measured with a micrometer (IP-54, Mitutoyo Co., Ltd., Kawasaki, Japan) with a precision of ± 1 μm.

Preparation of the tonoplast-free cell

The tonoplast-free cell was prepared by perfusing the vacuole with the EGTA medium. The procedure to make the tonoplast-free cell by means of the vacuolar perfusion was described in detail by Tazawa and Shimmen (1987). Since in preparation of the tonoplast-free cell both cell ends were cut, the cell length was shortened considerably. This eventually affects *L*p. Therefore, a control experiment was done to check whether the cell shortening affects *L*p or not. First, *L*p of a cell was measured. Then, the cell underwent the cell operation of cutting and ligation but without the vacuolar perfusion. Finally, *L*p of the operated cell was determined. No difference

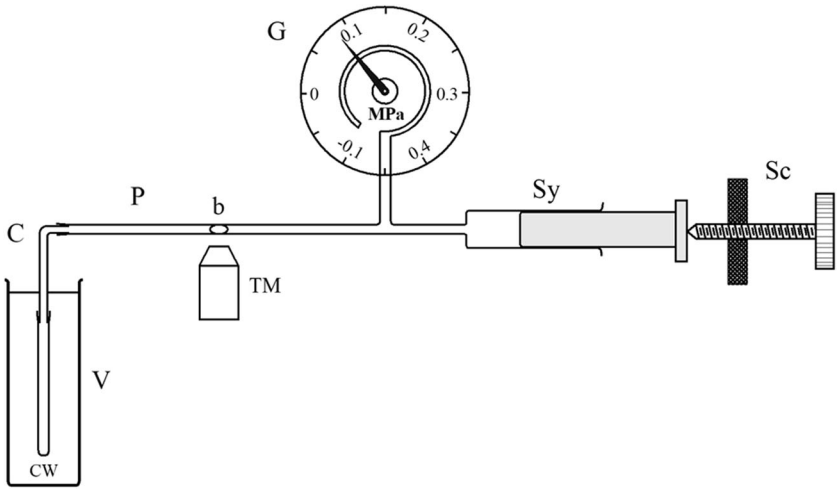

Fig. 2 The apparatus for measurement of water flow across the cell wall tube (cw) that is immersed in the water-filled vessel (V). The cell wall tube is connected to the measuring pipette (P) through the glass capillary (C). The flow of water across the cell wall tube is induced by applying hydrostatic pressure inside the pipette and the syringe (Sy). The pressure indicated by the pressure gauge (G) is changed by the movement of the screw (Sc). The water flow is indicated by the shift of the air bubble (b) and followed by the ocular micrometer in the traveling microscope (TM)

in Lp values was found between the cells (13 cells) before (1.74 ± 0.32 pm s^{-1} Pa^{-1}) and after (1.74 ± 0.31 pm s^{-1} Pa^{-1}) the cell operation. In conclusion, shortening of the cell caused by the cell operation has no effect on the hydraulic resistance or the hydraulic conductivity of the cell.

Determination of the hydraulic resistance of the membrane (Lp_m^{-1}), the plasma membrane (Lp_{pm}^{-1}), and the tonoplast (Lp_{tp}^{-1})

After measurement of the Lp^{-1} of a cell, the vacuole of the cell was perfused with the EGTA medium to remove the tonoplast. The Lp^{-1} of the tonoplast-free cell, ${}^{tpf}Lp^{-1}$, was measured. Then, the cell wall tube was prepared and its $Lp_w{}^{-1}$ was determined. $Lp_m{}^{-1}$ was calculated from Lp^{-1} and $Lp_w{}^{-1}$ using Eq. (1). Since the hydraulic resistance of the tonoplast-free cell, ${}^{tpf}Lp^{-1}$, is the sum of $Lp_w{}^{-1}$ and $Lp_{pm}{}^{-1}$, $Lp_{pm}{}^{-1}$ was calculated using Eq. (9).

$$ {}^{tpf}Lp^{-1} = Lp_w{}^{-1} + Lp_{pm}{}^{-1} \quad (9) $$

Then, $Lp_{tp}{}^{-1}$ was obtained from $Lp_m{}^{-1}$ and $Lp_{pm}{}^{-1}$ using Eq. (2).

In the present method, $Lp_w{}^{-1}$ was not measured in the normal cell but in the same cell after the tonoplast had been removed. The same value of $Lp_w{}^{-1}$ was used for the calculation of $Lp_m{}^{-1}$ of the normal cell under the assumption that $Lp_w{}^{-1}$ was not changed by making the cell tonoplast free. The validity of this assumption was supported by the fact that the dependence of $Lp_w{}^{-1}$ on the thickness of the cell wall (d) remained practically the same between the normal cells and the tonoplast-free cells (Fig. 6).

Statistics

Student's t tests were conducted to test the significant difference between the data from different groups. Significance of regression lines and lack of fit were analyzed with JMP software.

Results

Hydraulic resistances of the cell wall (Lp_w^{-1}) and the plasma membrane (Lp_m^{-1}) in young and old cells

A way of testing the dependence of $Lp_w{}^{-1}$ and $Lp_m{}^{-1}$ on the age of the cells is to compare $Lp_w{}^{-1}$ and $Lp_m{}^{-1}$ between the cells of different ages. In the main axes of a shoot of *Chara*, the age of the internodal cell increases basipetally, from the tip to the base. In the present experiment, cells of different ages were isolated from *Chara* shoots and their Lp^{-1} and $Lp_w{}^{-1}$ were measured. The $Lp_m{}^{-1}$ was calculated from Lp^{-1} and $Lp_w{}^{-1}$ using Eq. (1).

The alga *Chara corallina* used in the present study was cultivated outdoors. The alga is dormant in winter. Internodal cells at the primordial stage start to grow in spring. The cell wall of young intermodal cells is markedly thinner than that of winter-spent older cells.

To compare the values of Lp^{-1}, $Lp_w{}^{-1}$, and $Lp_m{}^{-1}$ between young and old cells, *Chara* shoots were taken from a culture bucket in early June (Fig. 3). At the tips of the main axes and branches, young growing cells were found, and at their bases, winter-spent old cells were found. Young cells in the spring could be easily discriminated from old cells by the difference of colors, since young cells looked pale green and old cells

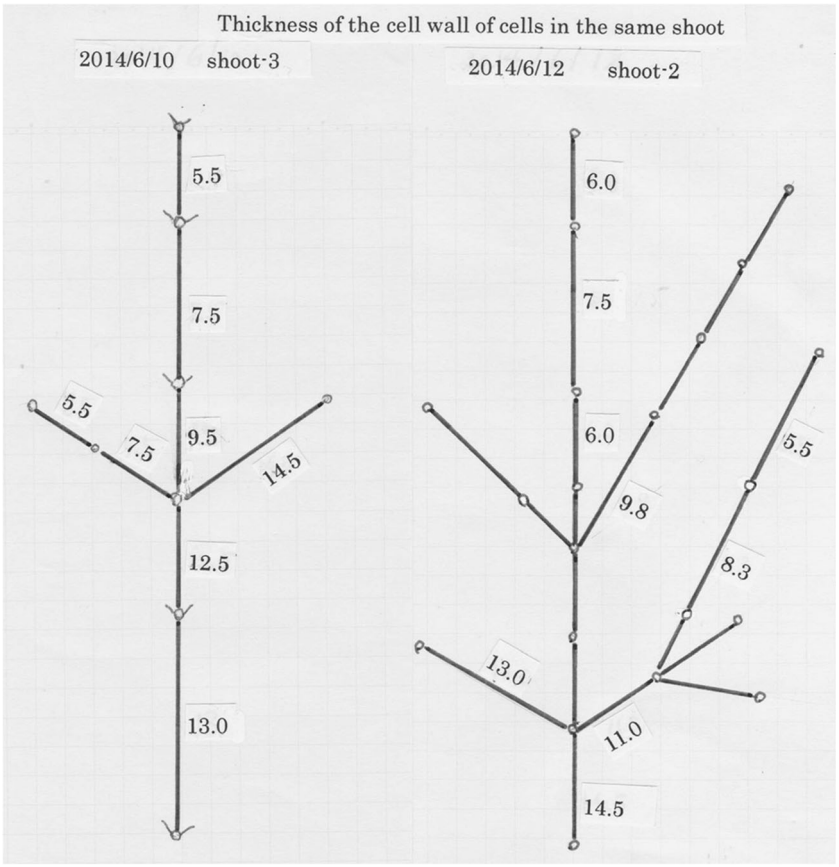

Fig. 3 The pictures showing schematically two *Chara* shoots isolated in June from a culture bucket. The thickness of the cell wall (in μm) is shown beside each intermodal cell

looked dark green. Pale green and dark green cells are designated as spring-grown cells or sg-cells (51 ± 7 (SD) mm in length) and winter-spent cells or ws-cells (54 ± 7 (SD) mm), respectively. The two groups of cells with different ages are characterized by the difference in the wall thickness (d). Namely, d of sg-cells was significantly smaller than that of ws-cells (Fig. 4). Then, the average values of Lp^{-1}, $Lp_w{}^{-1}$, and $Lp_m{}^{-1}$ were compared between sg-cells and ws-cells. The results are shown in Fig. 5. The hydraulic resistance of the membrane $Lp_m{}^{-1}$ is also dependent on the cell age showing that $Lp_m{}^{-1}$ of sg-cells is significantly less than that of ws-cells. There is no difference in the specific hydraulic resistance of the cell wall defined as $Lp_w{}^{-1}/d$ between sg ($r_w = 0.026 \pm 0.006$ (SD) $\times 10^{18}$ m^{-2} s Pa) and ws cells ($r_w = 0.026 \pm 0.005$ (SD) $\times 10^{18}$ m^{-2} s Pa).

Hydraulic resistance of the cell wall ($Lp_w{}^{-1}$) in relation to the thickness of the cell wall (d)

Using the cell wall tubes prepared from *Chara* cells, $Lp_w{}^{-1}$ and d were measured. In Fig. 6, open circles are plots of $Lp_w{}^{-1}$ against d in normal cells. The regression line is expressed with an equation of $Lp_w{}^{-1} = 0.024d + 0.043$. A question arises whether r_w may eventually be affected by making the cell tonoplast free, since a Ca^{2+}-chelating agent EGTA was contained in the perfusion medium. In order to check this question, $Lp_w{}^{-1}$ of tonoplast-free cells was plotted against d (filled circles in Fig. 6). The regression line is expressed with an equation of $Lp_w{}^{-1} = 0.03d - 0.016$. The regression lines for

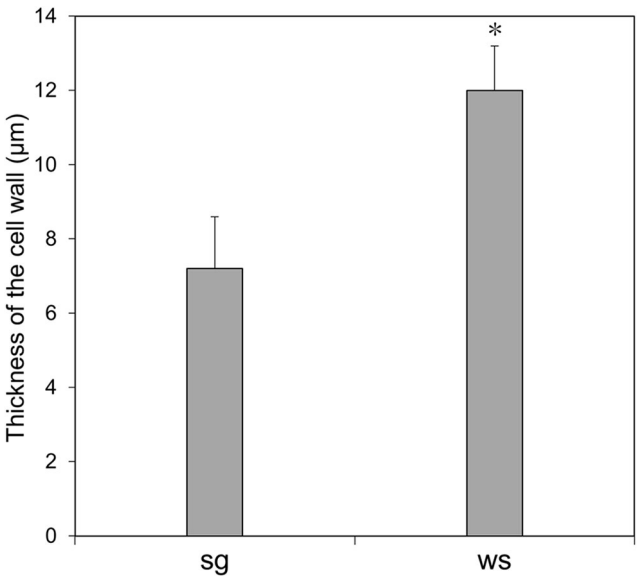

Fig. 4 Thickness of the cell wall in spring-grown cells (sg-cells) and winter-spent cells (ws-cells). Each bar shows the mean ± SD ($n = 6$ for sg and $n = 4$ for ws). $^*p < 0.001$

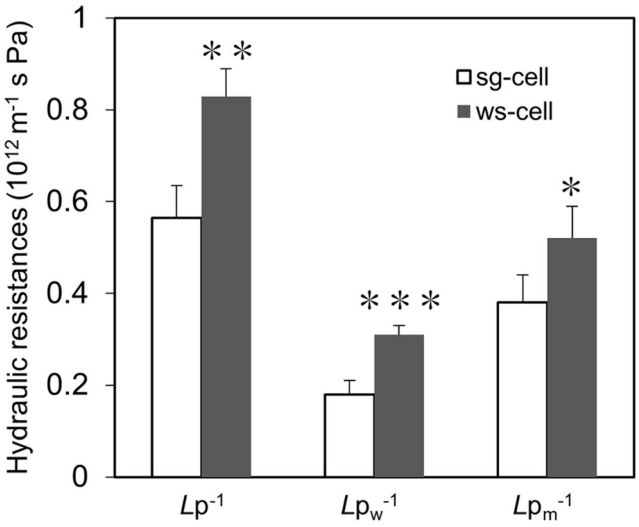

Fig. 5 Hydraulic resistances of the cell (Lp^{-1}), the cell wall (Lp_w^{-1}), and the membrane (Lp_m^{-1}) of *Chara* cells in relation to the thickness of the cell wall (in μm). Cells were classified into two groups, young spring-grown (sg, white bars) cells with the average wall thickness of 7.8 μm and old winter-spent (ws, black bars) cells with the average wall thickness of 12.0 μm. Hydraulic resistances are in 10^{12} m^{-1} s Pa. Each bar shows the mean ± SD ($n = 6$ for sg and $n = 4$ for ws). $^{*}p < 0.01$, $^{**}p < 0.001$, $^{***}p < 0.0001$

the normal and the tonoplast-free cells were subjected to the lack of fit test in JMP. The test shows that both equations are not significantly different, suggesting that the hydraulic resistance of the cell wall was not affected by the operation of making the cell tonoplast free.

Hydraulic resistances of the plasma membrane (Lp_{pm}^{-1}) and the tonoplast (Lp_{tp}^{-1}) versus the thickness of the cell wall (*d*)

In order to know how the hydraulic resistances of the plasma membrane (Lp_{pm}^{-1}) and the tonoplast (Lp_{tp}^{-1}) are related to the cell age, both Lp_{pm}^{-1} (Fig. 7a, filled circles) and Lp_{tp}^{-1} (Fig.7a, open circles) are plotted against the thickness of the cell wall (*d*) which was adopted as a marker of the cell age. Figure 7a shows that both Lp_{pm}^{-1} and Lp_{tp}^{-1} are dependent on cell age using *d* as a proxy and that the former is distinctly higher than the latter.

The ratio of $Lp_{pm}^{-1}/Lp_{tp}^{-1}$ for each *d* can be calculated from the regression lines shown in Fig. 7a. For 5 μm of *d*, the ratio is 30. With increase in *d*, the ratio decreases. For 11 μm of *d*, the ratio amounts to 5.0. This is because of the fact that the rate of increase in Lp_{pm}^{-1} with aging is less than the rate of increase in Lp_{tp}^{-1}, suggesting that the tonoplast ages faster than the plasma membrane so far as the hydraulic resistance is concerned. This conclusion is supported by a high correlation of hydraulic resistances between the plasma membrane and the tonoplast (Fig.7b).

Discussion

In the present study, the dependence of the hydraulic resistance (reciprocal of the hydraulic conductivity) of internodal cells of *Chara corallina* on cell age was studied. As an index of the cell age, the thickness of the cell wall was adopted based on the following facts: in the shoot of the *Chara* plant, growth occurs in young cells at the tip but not in old cells at the base of a shoot; and the average value of the wall thickness of spring-grown young cells (sg-cells) was significantly less than that of the winter-spent aged cells (ws-cells) (Fig.4).

The Lp_w^{-1} of sg-cells is distinctly smaller than that of ws-cells. In internodal cells of *Nitella flexilis*, Kamiya et al. (1962) found that Lp_w^{-1} is linearly dependent on the thickness of the cell wall (*d*). The same is true also for *Chara corallina*. A new finding in the present study is that Lp_m^{-1} was also correlated with the wall thickness (*d*). Lp_m^{-1} increased with an increase

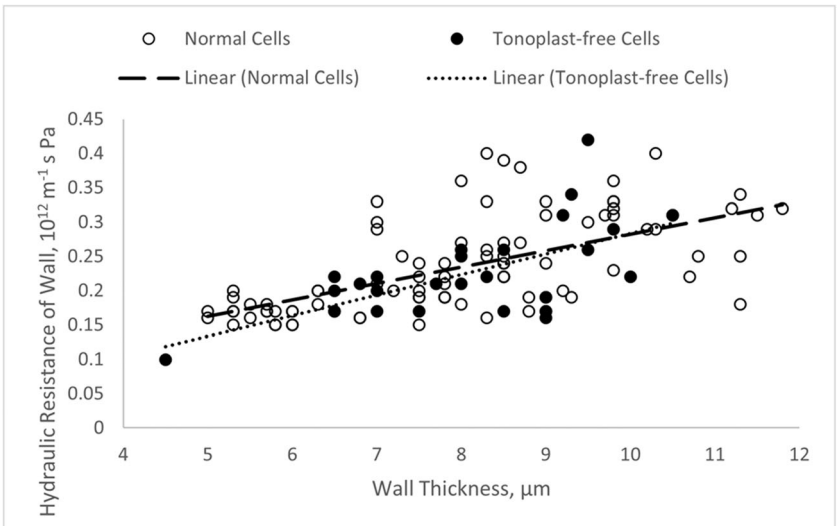

Fig. 6 Hydraulic resistance of the cell wall (Lp_w^{-1}) of normal intermodal cells (open circles) or tonoplast-free cells (filled circles) in relation to the thickness of the cell wall (*d*). A lack of fit test using JMP software shows that the linear regression lines for normal cells (dashed line; $Lp_w^{-1} = 0.024d + 0.043$; $p = 0.0001$) with $R^2 = 0.3791$, and that for tonoplast-free cells (dotted line; $Lp_w^{-1} = 0.03d - 0.016$; $p = 0.0007$) with $R^2 = 0.3849$ are not significantly different where $p = 0.2425$

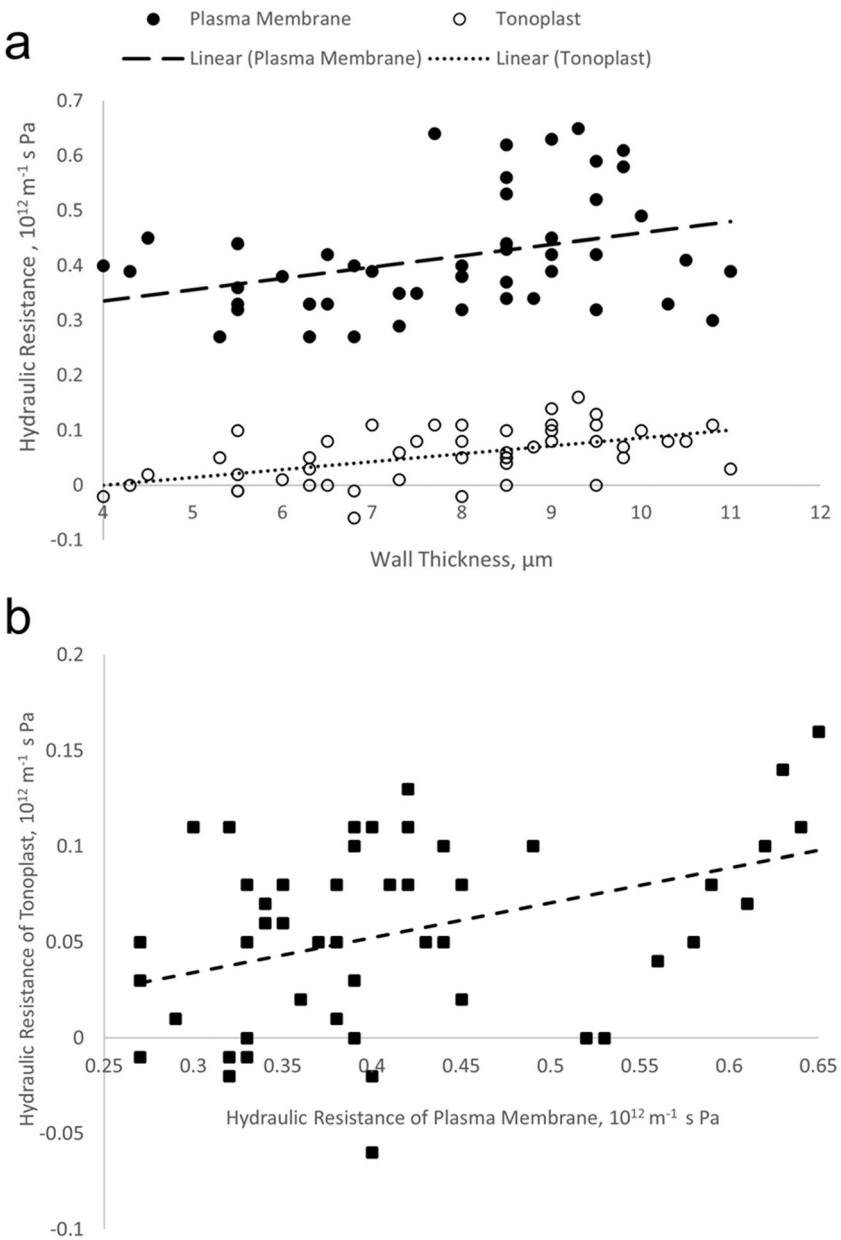

Fig. 7 **a** Hydraulic resistances of the plasma membrane (filled circles, Lp_{pm}^{-1}) and the tonoplast (open circles, Lp_{tp}^{-1}) in relation to the thickness of the cell wall (d). Linear regression lines for the plasma membrane (dashed line; $Lp_{pm}^{-1} = 0.02d + 0.26$) with $R^2 = 0.1218$, and for the tonoplast (dotted line; $Lp_{tp}^{-1} = 0.01d - 0.05$) with $R^2 = 0.2712$ are statistically significant, $p = 0.02$ and $p = 0.003$, respectively. **b** Relation between hydraulic resistances of the plasma membrane and the tonoplast. Linear regression line with $R^2 = 0.1526$ ($Lp_{tp}^{-1} = 0.18Lp_{pm}^{-1}$). The regression equation is significant ($p = 0.0055$)

in d (Fig.5). Since Lp_m^{-1} is composed of Lp_{pm}^{-1} and Lp_{tp}^{-1}, Lp_{pm}^{-1} and Lp_{tp}^{-1} were examined separately for their dependences on d which is an index of the cell age.

The present study is characterized by the method of measuring the hydraulic conductivities (Lp) or the osmotic water permeabilities (P_f) of the cell wall (Lp_w, P_{fw}), the plasma membrane (Lp_{pm}, P_{fpm}), and the tonoplast (Lp_{tp}, P_{ftp}) in an individual cell. On the other hand, many data on the respective membranes in higher plants were not obtained in individual cells but in membrane vesicles (Maurel et al. 1997; Niemietz and Tyerman 1997; Ohshima et al. 2001; Maurel et al. 2008). An elegant method for measuring the P_f of a single protoplast (P_{fpst}) isolated from the radish root cortex or the endodermis was developed by Suga et al. (2003). The method is based on the measurement of osmotic volume changes of a single protoplast. The average value of P_{fpst} amounted to 365 µm s^{-1}. The value was assumed to represent the value of the plasma membrane (P_{fpm}), since in radish P_{ftp} was found to be much higher than P_{fpm} (Ohshima et al. 2001). Using the same method of Suga et al. (2003), Murai-Hatano and Kuwagata (2007) prepared the protoplast from radish root cortex and measured the osmotic water permeability of the protoplast (P_{fpst}) and that of the vacuole (P_{ftp}). The vacuole was prepared through rupturing the protoplast by osmotic swelling induced by the transfer of the protoplast from 450 mOsm sorbitol solution to 250 or 300 mM sorbitol solution. From the values of P_{fpst} and P_{ftp}, the value of P_{fpm} was calculated. Thus, the data obtained represent P_{fpm} and P_{ftp} of an individual cell. Values of P_{fpm}

and P_{ftp} amounted to about 500 μm s^{-1} without a significant difference between the two. The value of P_{fpm} is about the same as that obtained by Suga et al. (2003) in radish root cells (365 μm s^{-1}).

Contrary to Murai-Hatano and Kuwagata (2007), Ohshima et al. (2001) reported that the P_{ftp} value is much higher than the P_{fpm} value. It is to be noticed that the test medium used by Ohshima et al. (2001) for the light-scattering measurement contained 1 mM EDTA, a Ca^{2+}-chelating agent, while the medium used by Murai-Hatano and Kuwagata (2007) for measurement of P_f of the vacuole was a simple sorbitol solution. Morillon and Lassalles (1999) isolated vacuoles from onion, rape, petunia, and red beet and measured P_f in solutions containing 1 mM Ca^{2+}, since the vacuoles were too fragile at more physiological Ca^{2+} concentrations. P_{ftp} values of the vacuoles obtained in solutions containing 1 mM Ca^{2+} were in the range of 200–1000 μm s^{-1} (Morillon and Lassalles 1999). In *Chara* cells the values of P_f was calculated from Lp using the equation $P_f = L\text{pRT}/V_w$ where V_w means molar volume of H_2O (Dainty 1963). In a young cell with $d = 5$ μm, Lp_{tp} is estimated from the regression line to be 83 pm s^{-1} Pa^{-1}. P_{ftp} converted from Lp_{tp} amounted to 11,200 μm s^{-1}. On aging or with increase in d to 8 and 11 μm s^{-1}, P_{ftp}, decreased to 2500 and 1400 μm s^{-1}. These values are markedly higher than those obtained in other plant materials, while P_{fpm} values for $d = 5$, 8, and 11 μm amounted to 380, 320, and 280 μm s^{-1}, respectively. These values are comparable to those obtained in other plant materials (Ramhaleo et al. 1999; Suga et al. 2003; Murai-Hatano and Kuwagata 2007). It is to be examined whether or not Lp of the isolated vacuole (Lp_{tp}) is dependent on the Ca^{2+} concentration of the ambient medium.

In order to see how Lp_{pm}^{-1} and Lp_{tp}^{-1} are related to the cell age, Lp_{pm}^{-1} and Lp_{tp}^{-1} are plotted against the wall thickness (d) (Fig. 7a).

It is to be noted that in a young cell with the wall thickness of 5.0 μm, the hydraulic resistance of the tonoplast (Lp_{tp}^{-1}) is 1/30 that of the plasma membrane (Lp_{pm}^{-1}). When the wall thickness increased to 8 and 11 μm, Lp_{tp}^{-1} increased greatly (4.5 and 8 times), while Lp_{pm}^{-1} changed little (1.2 and 1.4 times) so that the ratio $Lp_{tp}^{-1}/Lp_{pm}^{-1}$ became less significant (1/8 and 1/5). An extremely high hydraulic conductivity of the tonoplast found in young cells is in accordance with the result reported by Kiyosawa and Tazawa (1977) that in *Chara* cells Lp was not affected by the removal of the tonoplast. Assumingly, *Chara* cells used by Kiyosawa and Tazawa (1977) were of young age.

Under the situation that the hydraulic conductivity of the tonoplast is much higher than that of the plasma membrane ($Lp_{tp} \gg Lp_{pm}$), the cytoplasm and the vacuole can behave against an osmotic shock almost as a single compartment, since the tonoplast does not act as a barrier to osmotic water flow. Tyerman et al. (1999) thoroughly discussed the physiological meaning of $Lp_{tp} \gg Lp_{pm}$ and explained it as such that "*the higher water permeability of the tonoplast allows the vacuole to buffer the cytoplasm rapidly, thereby preventing short-term volume changes in the cytoplasm that might otherwise damage the cytoskeleton and metabolism*." In *Chara corallina*, a high ratio of Lp_{tp}/Lp_{pm} is more distinct in young growing cells than in old cells. Young cells having a thin cell wall (a small value of d) and a low value of Lp_{pm}^{-1} are assumed to be more sensitive to osmotic perturbation than old cells having a thick cell wall (a large value of d) and a high value of Lp_{pm}^{-1}. But a very low value of Lp_{tp}^{-1} in young cells may prevent the damage which otherwise may occur in the cytoplasm due to external osmotic perturbation.

In a shoot of the alga *Chara corallina*, young cells have lower hydraulic resistances of the cell wall and the membrane than old cells. The increase in the hydraulic resistance of the cell wall with aging is caused by the thickening of the cell wall. But the increase in hydraulic resistances of the plasma membrane and the tonoplast may be accounted for by a decrease of the density of aquaporins in these membranes, since most of the hydraulic conductivity of *Chara* cells is assumed to be via Hg-sensitive water channels (Tazawa et al. 1996). A positive relationship between aquaporin contents and hydraulic conductivities of the plasma membrane and the tonoplast was demonstrated in radish and a CAM plant (Ohshima et al. 2001).

So far as the authors know, this is the first report dealing with the dependence of the hydraulic resistances of the plasma membrane and the tonoplast of individual cells on the cell age. In this respect, an interesting paper was published by Kaldenhoff et al. (1995) which deals with the expression of a plasma membrane aquaporin gene *AthH2* (present name: *AtPIP1;2)* in *Arabidopsis thaliana*. Using the GUS reporter gene, a pronounced expression of *AthH2* was observed in tissues where cell elongation and/or cell differentiation takes place. In the root, the expression was high in the elongating region and faded away in the region where the elongation growth had ceased. The result suggests that the cell water permeability in the root is related to the cell age.

On the other hand, Ramhaleo et al. (1999) measured the osmotic water permeability (P_f) of protoplasts prepared from roots of young seedlings of rape, wheat, and maize. P_f amounted to 1~1000 μm s^{-1}. The P_f value of protoplasts isolated from 2 to 5 day old roots (maize, wheat, and rape) revealed a dramatic increase in P_f during root development, a shift of P_f from 10 in 2-day old roots to 500 μm s^{-1} in 3-to 5-day-old roots. A dramatic increase in the plasma membrane P_f value occurred within 48 h. At a glance, the result indicates that P_f increases with aging. However, a wide dispersion of P_f values observed in protoplasts from 3- to 5-day-old roots may suggest that the protoplasts used may be derived from cells of different tissues and ages. It is necessary to identify the tissue from which the protoplasts were prepared.

Using cells of *Chara corallina*, Wayne et al. (1994) found variability of Lp^{-1} values among cells with different cell surface areas and between cells isolated from vegetative plants and cells isolated from reproductive plants. It is interesting to examine whether or not these variabilities are related to the thickness of the cell wall which is supposed to be an index of the cell age. Another problem is whether or not the age dependence of $Lp_{pm}{}^{-1}$ and $Lp_{tp}{}^{-1}$ found in *Chara* would be observed in other plants.

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