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Hydraulic Conductivity of Tonoplast-Free *Chara* Cells

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Summary. This study is the first trial to measure the osmotic water permeability or the hydraulic conductivity of the plasmalemma alone of a plant cell. For this purpose tonoplast-free cells were prepared from internodal cells of *Chara australis* and their hydraulic conductivities were measured by the transcellular osmosis method.

The transcellular hydraulic conductivity did not change after removing the tonoplast. The transcellular hydraulic conductivity of the tonoplast-free cells was dependent on the internal osmotic pressure as is the case in the tonoplast-containing normal cells. The hydraulic conductivities for both endosmosis and exosmosis of the tonoplast-free cells were equal to respective values of the normal cells. Consequently the ratio between the inward and outward hydraulic conductivities did not change due to the loss of the tonoplast. The results indicate that the resistance of the tonoplast to water flow is negligibly small as compared with that of the plasmalemma and further that the tonoplast is not a factor responsible for the direction-dependency of hydraulic conductivity. The hydraulic conductivity of the plasmalemma is invariable for wide variations of K^+ and Ca^{2+} in the cytoplasm.

The osmotic water permeability or hydraulic conductivity (L_p) of plant cells is determined by measuring the volume of water moved between the vacuole and the external medium. The reciprocal of hydraulic conductivity or the hydraulic resistance ($L_p{}^{-1}$) is the sum of the resistance of the cell wall and of the resistances of the transport barriers located inside the protoplasmic envelope which are inserted in series in the water transport process between the vacuole and the external medium. In *Nitella flexilis* the hydraulic resistance of the cell wall is $^1/_2$–$^1/_4$ that of the protoplasmic layer according to the thickness of the cell wall which varies mostly between 4 and 8 μm (Kamiya, Tazawa & Takata, 1962). As for the protoplasmic layer, the contributions of the endoplasmic layer and the chloroplast layer to the total resistance are so small that it cannot be determined quantitatively (Tazawa & Kamiya, 1965; Kiyosawa & Tazawa, 1972*b*). Similar results were obtained for epidermal cells of *Allium cepa* (Url, 1971). Thus, it was concluded that in Characeae cells the two cytoplasmic membranes, plasmalemma and tonoplast, are the main sites of the resistance to osmotic water flow. To know the contribution of each membrane to the total resistance, it is necessary to measure L_p of each membrane separately. Using onion epidermal cells, Url (1971) measured L_p of the tonoplasts which were formed accidentally from the plasmolyzed cells by subjecting them repeatedly to plasmolysis and deplasmolysis.

Recently, Tazawa, Kikuyama and Shimmen (1976) succeeded in removing the tonoplast by perfusing the vacuole of Characeae cells with a solution containing ethyleneglycol-bis (β-amino-ethylether)-N, N′-tetraacetic acid (EGTA). Thus, it has become possible to measure L_p of the plasmalemma alone. When L_p of the plasmalemma is known, L_p of the tonoplast can be calculated from L_p of the protoplasmic layer of the intact cell having both plasmalemma and tonoplast, since the plasmalemma and tonoplast are inserted in series as barriers against the osmotic water flow.

In the present study we tried (i) to determine L_p of the plasmalemma and tonoplast separately and (ii) to check whether some membrane characteristics found in the intact cell having the two membranes are also observable in the cell having only plasmalemma; these characteristics are dependency of L_p on the internal osmotic pressure (Kiyosawa & Tazawa, 1972*a*) and on the direction of osmosis (Kamiya & Tazawa, 1956; Dainty & Hope, 1959; Dainty & Ginzburg, 1964; Tazawa & Kamiya, 1965, 1966; Kiyosawa & Tazawa, 1973; Tazawa & Kiyosawa, 1973; Steudle & Zimmermann, 1974).

Materials and Methods

Plant Material

Internodal cells of *Chara australis* were used throughout the experiments. The internodes were isolated from adjacent cells and stored in a petri dish with artificial pond water (APW: 0.1 mM KCl, 0.1 mM NaCl, and 1 mM $CaCl_2$).

Measurement of Transcellular Hydraulic Conductivity L_p

The internode (*n*) was set in a double-chamber osmometer in such a manner that one half of the cell was in chamber *A* and the other equal half in chamber *B* (Fig. 1). At first, both chambers were filled with APW. Transcellular osmosis was then induced by replacing APW in chamber *B* with APW containing 200 mM sorbitol. The volume of water transported transcellularly, which is indicated by the movement of air-bubble *C* in the capillary of the osmometer, was measured at internvals of 6 sec. Transcellular

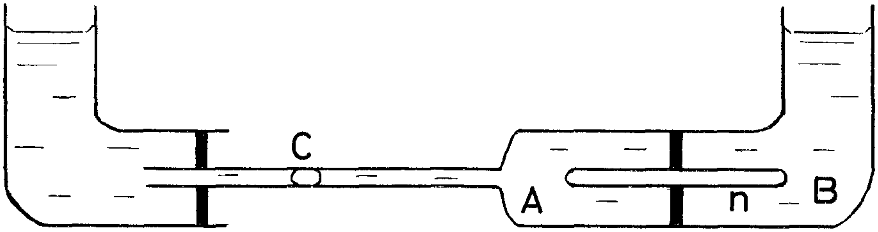

Fig. 1. Diagrammatic representation of the double chamber osmometer for measuring transcellular water flow. An internodal cell (n) is partitioned into two chambers A and B. An air bubble C in the capillary serves as an indicator of water flow from A to B or *vice versa*

hydraulic conductivity L'_p defined by Tazawa and Kamiya (1966) was calculated from the equation

$$L'_p = \frac{(dv/dt)_i}{(A/2)\pi_0} \tag{1}$$

where A is the sum of the surface areas of the cell parts in chamber A and B, π_o the osmotic pressure of the solution in chamber B, $(dv/dt)_i$ the initial rate of transcellular osmosis, a rate which was obtained from the volume of water driven transcellularly between the first 6 and 18 sec.

Measurement of Hydraulic Conductivities for Endosmosis $(L_p)_{\mathrm{en}}$ *and for Exosmosis* $(L_p)_{\mathrm{ex}}$ *of the Protoplast*

To determine the degree of direction-dependency of L_p, L_p of the protoplasmic layer on the endosmosis side $(L_p)_{\mathrm{en}}$ and that on the exosmosis side $(L_p)_{\mathrm{ex}}$ were determined after the method of Tazawa and Kiyosawa (1973). Namely, $(L_p)_{\mathrm{en}}$ and $(L_p)_{\mathrm{ex}}$ were calculated from the following equations:

$$(L_p)_{\mathrm{en}} = \frac{1}{\pi_{v\mathrm{en}} - P + P_{w\mathrm{en}}} \frac{\dot{v}}{A_{\mathrm{en}}} \tag{2}$$

$$(L_p)_{\mathrm{ex}} = \frac{1}{\pi_0 - \pi_{v\mathrm{ex}} + P + P_{w\mathrm{ex}}} \frac{\dot{v}}{A_{\mathrm{ex}}} \tag{3}$$

where π_v is the osmotic pressure of the cell sap calculated both from the original osmotic pressure of the cell sap and the volume of water transported transcellularly; P is the turgor pressure measured with the turgor balance (Tazawa, 1957); P_w is the pressure gradient induced by water flow across the cell wall; $\dot{v}$ is the rate of transcellular osmosis at time t obtained from the difference between the volumes of water transported transcellularly until $(t-6)$ sec and $(t+6)$ sec; A is the surface area of the cell; suffixes en and ex represent the endosmotic and exosmotic sides, respectively. Measurements of the volume of water transported and the turgor pressure at the initial phase of transcellular osmosis were carried out every 6 sec after the onset of osmosis, i.e., 6, 12, 18, 24 and 30 sec. Then $(L_p)_{\mathrm{en}}$ and $(L_p)_{\mathrm{ex}}$ were calculated at intervals of 6 sec.

Preparation of Tonoplast-Free Cells and Cells with Abnormal Osmotic Pressures

To remove the tonoplast the cell sap was replaced by a solution containing 3 or 5 mM EGTA and 5 mM tris-maleate buffer (adjusted at pH 7.0 with KOH) by the vacuolar perfusion (Tazawa *et al.*, 1976). The osmotic pressures of the solutions were adjusted or altered by sorbitol. According to Tazawa *et al.* (1976), cells whose cell saps are replaced with the media of low ionic strengths containing 3 or 5 mM EGTA lose the tonoplast within 30 min after perfusion. Thus, in our experiments cells were kept in APW for at least 30 min before measuring hydraulic conductivity. A reliable indicator for the loss of the tonoplast was the appearance of endoplasmic fragments of various sizes in the vacuole. The protoplasmic streaming continued in such cells at a rate which was slightly lower than normal (Tazawa *et al.*, 1976).

The natural cell sap of *Nitella* or *Chara* cells was condensed or diluted by the method of transcellular osmosis (Kamiya & Kuroda, 1956). By ligation and amputation of an internodal cell after 15–20 min of transcellular osmosis, two cell fragments were obtained, one with the cell sap of higher osmotic pressure (*NH*) and the other with the cell sap of lower osmotic pressure (*NL*).

All the experiments were performed at 19–21 °C.

Results

Table 1. shows L'_p of the cells before and after the cell sap was replaced with the EGTA-containing media. The concentration of free Ca^{2+} in cells with media *I* and *II* after the loss of the tonoplast can be calculated under the assumption that all the calcium in the protoplasm distributes homogenously in the whole cell space. Since the concentration of endogenous calcium on the basis of the whole cell volume is 0.3 mM (Tazawa *et al.*, 1976), the concentrations of free Ca^{2+} in the tonoplast-free cells with media *I* and *II* are calculated to be 2.3×10^{-8} M and 2.9×10^{-6} M, respectively, if the apparent binding constant between Ca^{2+} and EGTA is assumed to be 4.83×10^{6} M^{-1} (Jewell & Rüegg, 1966). From the Table, it is clear that no differences were detected between L'_p of the normal cells having the tonoplast and that of the cells lacking the tonoplast. The concentration of free Ca^{2+} in the cell had no effect on the magnitude of L'_p. Procedures of ligation and amputation did not affect L'_p (Cells 7 and 8 in Table 1).

Table 2. shows changes in L'_p of the cells having the tonoplast (saps *NH* and *NL*) and those of the cells lacking the tonoplast (media *III*, *IV* and *V*) by increasing (sap *NH*; media *III* and *IV*) or decreasing (sap *NL*; medium *V*) the osmotic pressure of the internal medium. L'_p of the cells having the hypotonic media was larger than that of the same cells before the operation irrespective as to whether the tonoplast was present (sap *NL*) or absent (medium *V*), while hydraulic conductivity of the cells having the hypertonic media (media *III* and *IV*; sap *NH*)

Table 1. Transcellular hydraulic conductivity (L'_p) of *Chara* cells before and after removing tonoplast with isotonic artificial cell saps

	Cell sap composition (mM)	Cell No.	Before replacement			After replacement		
			OP M	L'_p pm sec^{-1} Pa^{-1}	%	OP M	L'_p pm sec^{-1} Pa^{-1}	%
I	5 tris-maleate	1	0.25	0.87	100	0.27	0.81	93
	3 EGTA	2	0.27	0.72	100	0.26	0.67	93
	12 KOH	3	0.29	0.64	100	0.30	0.66	103
	310 sorbitol	Average	0.27	0.74	100	0.28	0.71	96
II	5 tris-maleate	4	0.30	0.51	100	0.30	0.54	106
	3 EGTA	5	0.28	0.79	100	0.26	0.74	94
	2.49 $CaCl_2$	6	0.28	0.77	100	0.25	0.79	103
	17 KOH	Average	0.29	0.69	100	0.27	0.69	100
	300 sorbitol							
			Before operation			After operation		
	Natural cell sap	7	–	0.67	100	–	0.66	99
	(only ligation	8	–	0.66	100	–	0.66	100
	and amputation)	Average	–	0.67	100	–	0.66	100

OP: osmotic pressure of the cell sap expressed in equivalent molarity of sorbitol. Concentrations of free calcium in the cells with media *I* and *II* are 2.3×10^{-8} M and 2.9×10^{-6} M, respectively (Tazawa *et al.*, 1976). Transcellular osmosis was induced by the osmotic gradient of 200 mM sorbitol.

was smaller than that of the same cells before the operation. Furthermore, Table 2 shows that the degree of increase or decrease of hydraulic conductivity by decrease or increase of the internal osmotic pressure is not dependent on the presence of the tonoplast but on the magnitude of modification of the internal osmotic pressure.

Table 3. shows $(L_p)_{en}$ and $(L_p)_{ex}$ of normal and tonoplast-free cells which were measured by transcellular osmosis induced by the osmotic gradient of 400 mM sorbitol. There is an equally significant difference between $(L_p)_{en}$ and $(L_p)_{ex}$ in the two types of cells. The polarity ($\rho_p = (L_p)_{en}/(L_p)_{ex}$) found in normal cells is therefore attributed to the characteristics of the plasmalemma.

Discussion

The fact that no difference in L_p between the tonoplast-free cells and the normal cells was found may be interpreted in two ways: first,

Table 2. Effects of lower and higher osmotic pressures of artificial and natural cell saps on transcellular hydraulic conductivity (L'_p) of the normal and tonoplast-free *Chara* cells

	Cell sap composition (mM)	Cell No.	Before replacement			After replacement		
			OP M	L'_p pm sec^{-1} Pa^{-1}	%	OP M	L'_p pm sec^{-1} Pa^{-1}	%
III	5 tris-maleate	9	0.30	0.46	100	0.64	0.27	61
	3 EGTA							
	12 KOH							
	640 sorbitol							
IV	5 tris-maleate	10	0.25	0.86	100	0.50	0.56	66
	3 EGTA	11	0.24	0.71	100	0.50	0.54	76
	12 KOH	Average	0.25	0.79	100	0.50	0.55	70
	500 sorbitol							
V	5 tris-maleate	12	0.25	1.01	100	0.18	1.06	105
	3 EGTA	13	0.26	0.84	100	0.18	0.96	114
	12 KOH	14	0.25	0.86	100	0.18	0.96	111
	170 sorbitol	Average	0.25	0.90	100	0.18	0.99	110
NH	Condensed natural	15	0.25	0.79	100	0.45	0.62	79
	cell sap	16	0.27	0.74	100	0.46	0.57	77
		17	0.28	0.69	100	0.48	0.54	79
		Average	0.27	0.74	100	0.46	0.58	79
NL	Diluted natural	18	0.26	0.71	100	0.13	0.79	111
	cell sap	19	0.26	0.82	100	0.14	0.92	112
		20	0.27	0.82	100	0.17	0.89	108
		Average	0.26	0.78	100	0.15	0.87	111

OP: osmotic pressure of the cell sap expressed in equivalent molarity of sorbitol. Transcellular osmosis was induced by the osmotic gradient of 200 mM sorbitol.

the tonoplast is so permeable to water that its removal changes L_p of the protoplast only to an extent which cannot be measured; second, L_p of the plasmalemma decreases just so much as to cover the possible increase in L_p of the protoplast due to loss of the tonoplast. The method developed by Tazawa *et al.* (1976) to remove the tonoplast by introducing media containing EGTA of low ionic strengths may cause some changes in the inner environment of the cytoplasm which the inner surface of the plasmalemma faces. However, judging from the facts that protoplasmic streaming occurred at nearly the normal rate and that the cell generated action potentials (Tazawa *et al.*, 1976; Shimmen, Kikuyama & Tazawa, 1976), these changes do not seem to cause serious modifications of the motile system and the plasmalemma. Since the rate of the proto-

Table 3. Hydraulic conductivities for endosmosis $(L_p)_{en}$ and exosmosis $(L_p)_{ex}$ and polarity $(\rho_p = (L_p)_{en}/(L_p)_{ex})$ of the protoplasmic layers of the normal and tonoplast-free *Chara* cells

	t^a sec	$(L_p)_{en}$ pm sec^{-1} Pa^{-1}	$(L_p)_{ex}$ pm sec^{-1} Pa^{-1}	ρ_p
Normal cells ($n^b=3$)	6	2.14±0.25	1.02±0.05	2.1±0.1
	12	2.35±0.13	1.23±0.10	1.9±0.1
	18	2.25±0.08	1.16±0.02	2.0±0.1
	24	2.20±0.23	1.13±0.05	1.9±0.1
	Average	2.24±0.05	1.13±0.05	2.0±0.1
Tonoplast-free cells ($n^b=5$)	6	2.27±0.17	1.13±0.02	2.0±0.2
	12	2.05±0.10	1.10±0.07	1.9±0.1
	18	2.00±0.10	1.07±0.08	1.9±0.1
	24	1.99±0.08	1.08±0.08	1.8±0.1
	Average	2.08±0.07	1.09±0.02	1.9±0.0

Transcellular osmosis was induced by the osmotic gradient of 400 mM sorbitol.

[a] *t* represents the time elapsed after onset of transcellular osmosis. $(L_p)_{en}$ and $(L_p)_{ex}$ were calculated from the values of v, P, P_w and $\dot{v}$ at t by Eqs. (2) and (3). Values are given with ±SEM.

[b] n represents the number of cells used.

plasmic streaming decreases conspicuously in the concentration range of Ca^{2+} above 10^{-6} M in *Chara corallina* (Williamson, 1975), it is a reasonable assumption that the concentration of free Ca^{2+} in the cytoplasm *in situ* may be around 10^{-7} M or less. The fact that L_p was not changed by increasing concentration of free Ca^{2+} from 2.3×10^{-8} M to 2.9×10^{-6} M indicates that changes in concentration of the cytoplasmic calcium due to loss of the tonoplast do not modify the plasmalemma so far as L_p is concerned.

A marked change in K^+-concentration of the cytoplasm does not change L_p. Assuming that after loss of the tonoplast the cytoplasmic K^+-ions disperse homogenously in the cell, the concentration of K^+ in the cell is estimated to be 20 mM when EGTA-sap *I* is used and 25 mM when EGTA-sap *II* is used (*cf.* Tazawa *et al.*, 1976). These concentrations are $^1/_5$–$^1/_4$ the concentration of K^+ in the cytoplasm of normal cells which is 112 mM (Tazawa, Kishimoto & Kikuyama, 1974). L_p of the tonoplast-free cells with low cytoplasmic K^+-concentrations was almost equal to that of the normal cell (Table 1). The same is true also between the cells with EGTA-sap of higher osmotic pressure (*IV* in Table 2) and the cells with the normal cell sap of higher osmotic pressure (*NH* in Table 2). The K^+-concentration of the former cells is estimated to be about 23 mM, while that of the cytoplasm of the latter cells is estimated to be about 191 mM. Thus, the K^+-concentration and ionic strength of the cytoplasm in contact with the inner surface of the plasmalemma have little effect on water permeability. All these facts suggest that the first interpretation that the tonoplast is far more permeable to water than the plasmalemma is the more likely. The fact that no significant difference was observed in both $(L_p)_{\mathrm{en}}$ and $(L_p)_{\mathrm{ex}}$ and consequently in the polarity $(\rho_p=(L_p)_{\mathrm{en}}/(L_p)_{\mathrm{ex}})$ between the intact cells and the tonoplast-free cells means that the site responsible for the direction-dependent hydraulic conductivity is the plasmalemma.

Similar conclusions about the site of main resistances to water permeation in higher plant cells were advanced by Huber and Höfler (1930, p. 448) and supported by experimental evidence that the tonoplast of the inner epidermal cell of the *Allium cepa* bulb scale has the hydraulic conductivity which is about 100 times higher than the hydraulic conductivity of the intact protoplast (Url, 1971).

In our previous paper (Tazawa & Kiyosawa, 1973; Kiyosawa & Tazawa, 1973), we demonstrated that polar hydraulic conductivity occurs in the initial phase (about 6 sec) of transcellular osmosis; (ii) that the endosmotic and exosmotic hydraulic conductivities are nearly constant at least during the first 54 sec; (iii) that the degree of polarity depends on the osmotic pressure of the external solution on the exosmosis side which is equal to the driving force for transcellular osmosis at the start of osmosis.

In the present experiment it was established that the dependency of L_p of the plasmalemma of tonoplast-free cells on the osmotic pressure of the internal medium is almost equal to that of the normal cells on the osmotic pressure of the cell sap (Table 2). This fact may be accounted for in terms of hydration changes in the plasmalemma which is brought about by keeping equilibrium of the water potentials between the inner phase of the plasmalemma and the adjacent cytoplasmic gel phase. During transcellular osmosis both water flux and osmotic pressure of the cell sap on the endosmosis side gradually decreases with time, while the osmotic pressure on the exosmosis side increases. At 6, 12, 18 and 24 sec after onset of transcellular osmosis induced by 400 mM sorbitol, the osmotic pressure decreased on the endosmosis side and increased on the exosmosis side by about 3, 7, 9 and 12%, respectively. Increase in $(L_p)_{\mathrm{en}}$ due to decrease in the internal osmotic pressure and decrease in $(L_p)_{\mathrm{ex}}$ due to increase in the internal and external osmotic pressures at respective times are estimated from Eq. (6) in our previous paper (Kiyosawa & Tazawa, 1972*a*). From the estimated values of $(L_p)_{\mathrm{en}}$ and

$(L_p)_{\text{ex}}$ the polarity $\rho_p (= (L_p)_{\text{en}}/(L_p)_{\text{ex}})$ at 6, 12, 18 and 24 sec is calculated to be 1.4, 1.5, 1.5 and 1.5. On the other hand, the observed values of the polarity at 6, 12, 18 and 24 sec were 2.0, 1.9, 1.9 and 1.9, respectively.

To explain the discrepancy between the observed and estimated values, Kiyosawa and Tazawa (1973) postulated that at the start of transcellular osmosis the osmotic pressure of the cytoplasm of the endosmotic cell half $\pi_{c\text{en}}$ becomes lower than the osmotic pressure of the vacuole $\pi_{v\text{en}}$ by $\dot{v}A_{\text{en}}^{-1}[(L_p)^{\text{t}}_{\text{en}}]^{-1}$ and that of the exosmotic cell half $\pi_{c\text{ex}}$ becomes higher than $\pi_{v\text{ex}}$ by $\dot{v}A_{\text{ex}}^{-1}[(L_p)^{\text{t}}_{\text{ex}}]^{-1}$, where $\dot{v}$ is the rate of transcellular osmosis; A is the surface area of the cell; $[(L_p)^{\text{t}}]^{-1}$ is the hydraulic resistance of the tonoplast. If the hydraulic resistance of the tonoplast is high, $\pi_{c\text{en}}$ decreases leading to increase in L_p of the cell and $\pi_{c\text{ex}}$ increases resulting in decrease in L_p.

However, the present result showing that the tonoplast hardly acts as a barrier to water flow does not support the hypothesis. An alternative explanation for the difference between the observed and estimated polarity is that the water flow across the plasmalemma itself affects L_p of the plasmalemma.

There are other indications suggesting that the water flow itself affects the membrane. In *Nitella flexilis,* water inflow causes a rapid and large depolarization of the membrane sometimes accompanying action potentials. The membrane conductance increases in parallel with the magnitude of depolarization. On the exosmosis side, however, no signficant changes in membrane potential and membrane resistance are observed (*cf.* Tazawa, 1972). The osmosis-induced changes in electric properties are suppressed by increasing the internal osmotic pressure and stimulated by decreasing it (Hayama, Nakagawa and Tazawa; *unpublished*). The membrane becomes less permeable to water by heightening π_v and more permeable by lowering π_v (Tazawa & Kamiya, 1965; 1966; Kiyosawa & Tazawa, 1972*a*). These facts suggest that the response of the membrane to water flow is influenced greatly by the internal osmotic pressure. The different responses of the membrane according to direction of water flow may be partly attributed to the difference in directions of the change in the osmotic pressure of the gel cytoplasm (ectoplasm) which determines the hydration of the plasmalemma.

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