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Measurement of the Cytoplasmic and Vacuolar Buffer Capacities in *Chara corallina*[1]

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ABSTRACT

The cytoplasm and the vacuole were isolated from internodal cells of *Chara corallina* by using the intracellular perfusion technique, and their buffer capacities (β_i) were determined from the titration curves. The pH of the isolated vacuolar sap was 5.19 ± 0.029 (mean ± standard error). At this pH, β_i was minimal and amounted to 0.933 ± 0.11 millimoles H^+/pH unit/liter vacuolar sap. The pH of isolated cytoplasm was 7.22 ± 0.028. β_i was minimal in this pH region and amounted to 14.2 ± 0.80 millimoles H^+/pH unit/liter cytoplasm. When 1% (volume/volume) Triton X-100 was added to the cytoplasmic solution to permeabilize the subcellular organelles, the cytoplasmic pH increased to 7.32 ± 0.026, where β_i was 20.35 ± 2.66 millimoles H^+/pH unit/liter cytoplasm. This shows that alkaline subcellular compartments exist in the cytoplasm and also that the cytoplasmic pH before adding Triton X-100 may represent the cytosolic pH. These data indicate that the pH values of the cytoplasm and the vacuole are regulated at the values where the β_i values are minimal. This suggests that ATP- and inorganic pyrophosphate-dependent H^+ pumps in the plasma membrane and the tonoplast could efficiently regulate the pH of both cytoplasm and vacuole in *Chara* internodal cells.

In plant cells both cytoplasmic and vacuolar pH values are normally regulated within narrow ranges. For example, a large shift of the external pH (pH_o) from an acidic to a more alkaline region has little effects on the cytoplasmic pH (pH_c) in *Neurospora* (9) and *Chara* (16), and the vacuolar pH (pH_v) in *Chara* (4). Because the plasma membrane of characean cells is highly permeable to H^+ (2, 13), the intracellular pH must be actively regulated (10). In a plant cell, the cytoplasm occupies a rather restricted region between the plasma membrane and the tonoplast. Recent transport studies using isolated vesicles have demonstrated H^+-translocating ATPases located both in the plasma membrane and the tonoplast (11) and a H^+-translocating pyrophosphatase located in the tonoplast (6). These H^+ pumps may be involved in the pH regulation mechanism.

So far the contribution of the plasma membrane H^+ pump to intracellular pH regulation is assumed to be minor, because inhibition of the plasma membrane H^+ pump by vanadate brings about no change in pH_c (9). Thus, in contrast to animal cells (7), cellular metabolism is thought to be a main pH_i regulator in plant cells (9). Only a limited number of studies have been done on the contribution of tonoplast H^+ pumps to pH_i regulation, mainly due to the difficulty of directly accessing the tonoplast from either the cytoplasmic or the vacuolar side. The vacuolar perfusion technique developed by Tazawa (15) is a useful tool to directly control the vacuolar environment. Taking advantage of the vacuolar perfusion, Moriyasu *et al.* (4) investigated the role of the tonoplast H^+ pump in pH_v regulation. As inhibition of the H^+ pump by chemical agents, such as *N,N'*-dicyclohexylcarbodiimide, caused an alkalinization of the vacuole, they concluded that the H^+ pump participates in regulating the pH_v. To estimate this contribution, it is essential to know the β_i[2] of both the cytoplasmic and vacuolar compartments. In this report we separately isolated these compartments from *Chara corallina* cells, measured their β_i, and discussed their implications in pH_i regulation.

MATERIALS AND METHODS

Plant Material and Culture

Mature internodal cells of *Chara corallina* were used for all experiments. The alga was cultured in an air-conditioned room (25 ± 2°C) under illumination for 15 h a day as described previously (4). Internodal cells were cut from their neighboring cells and stored overnight in an artificial pond water containing 0.1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$, and 0.1 mM $MgCl_2$.

PH Measurement on Vacuolar Sap and Cytoplasm

The pH_c and pH_v were measured using a pH microelectrode (Micro combination pH probe MI-410, Microelectrodes, Inc.) as described previously (4). To collect the vacuolar sap, both cell ends were cut off and a glass microcapillary was inserted into the vacuole to collect the sap without contamination by cytoplasmic droplets (capillary method) (8). A microscope was used to check for the presence of contaminating cytoplasmic droplets. Contaminated samples were discarded.

The cytoplasm was collected by squeezing out the cell content with a cylindrical Teflon bar after perfusing the vacuolar space with a medium containing 100 mM KCl, 30 mM NaCl, and 10 mM $MgCl_2$. The pH of the medium was adjusted to 7.0 with KOH. This perfusion medium mimicked

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[2] Abbreviation: β_i, buffer capacity.

Table I. *pH Values of Natural Vacuolar Sap and Cytoplasmic Solution in Chara*

Solution	pH
Cytoplasm (−T)[a]	7.22 ± 0.028 (n = 13)
Cytoplasm (+T)	7.32 ± 0.026 (n = 13)
Vacuole	5.19 ± 0.029 (n = 6)

[a] (−T), (+T) are the cytoplasmic solution without or with the 1% Triton X-100.

the cytoplasmic K^+ concentration and the osmolality to avoid destruction of cell organelles. Vacuolar perfusion was performed after Tazawa (15). Perfusion of the vacuole with the perfusion medium of three times the cell volume removed more than 99% of the activity of acid phosphatase (data not shown). Thus we can neglect contamination of the natural vacuolar sap in the isolated cytoplasmic solution. The collected solutions from 4 to 6 cells (cytoplasmic solution) were combined so that a pH titration could be performed.

One hundred eighty μL of the vacuolar sap or the cytoplasmic solution was used for the pH titration. After the pH of a sample had stabilized, the titration was initiated by adding either 2.5 μL of 10 mM HCl in the acidic direction, or 5 μL of 10 mM KOH in the alkaline direction. These KOH solutions were standardized against a commercial 1 mol/L HCl calibration solution.

Subcellular organelles existing in the cytoplasm were permeabilized by adding 20 μL of a triton X-100 medium containing 10% (v/v) Triton X-100, 100 mM KCl, 30 mM NaCl, and 10 mM $MgCl_2$ (pH 7.0) to 180 μL of the cytoplasmic solution.

Chemicals

One mol/L hydrochloric acid was purchased from Wako Pure Chemical Industries LTD. (Osaka). All other chemicals used were of analytical grade.

RESULTS AND DISCUSSION

Measurements of pH_v and pH_c

Table I shows pH_v and pH_c values of internodal cells of *C. corallina*. Our pH_v of 5.19 is slightly higher than the value of 4.75 obtained with a microantimony electrode (4) but is similar to the value of 5.25 on the *Nitellopsis obtusa* with the ^{31}Pi-NMR (3). Our pH_c value of 7.22 (Table I) is slightly lower than those reported for *C. corallina* (7.5) obtained by the 5,5-dimethyloxazolidine-2,4-dione method (16) and for *Nitellopsis obtusa* (7.3) obtained by ^{31}P-NMR (3). When Triton X-100 was added to the cytoplasmic solution to permeabilize the organelles, the pH increased from 7.22 to 7.32 (Table I). This is not a direct effect of Triton X-100, because the pH of the Triton medium was 7.0. Furthermore, addition of 20 μL of the perfusion medium to the cytoplasmic solution caused a pH shift of only 0.01 unit. Thus the observed pH shift suggested the presence of subcellular compartments which are more alkaline than the cytosol. In other words, the cytoplasmic pH before adding Triton X-100 may represent the cytosolic pH.

Buffer Capacity of Natural Vacuolar Sap and Cytoplasm

Figure 1a shows a representative pH titration curve of the natural vacuolar sap. Figure 1b shows the calculated buffer capacity (β_{iv}). The points in Figure 1b represent the middle point of the pH ranges of 0.2 in the titration curve. β_{iv} was approximated from the slope, $\Delta H^+/\Delta pH$, between neighboring points. The β_{iv} is minimal (0.993 ± 0.11 mmol H^+/pH unit/L vacuolar sap) between 5.0 and 5.2, at a region corresponding to pH_v.

The representative pH titration curves of the cytoplasmic solution, one with and the other without Triton X-100, are shown in Figure 2a. From the curves, the buffer capacity of the cytoplasm (β_{ic}) with or without Triton X-100 was calculated as described above (Figure 2b). For the calculation, the

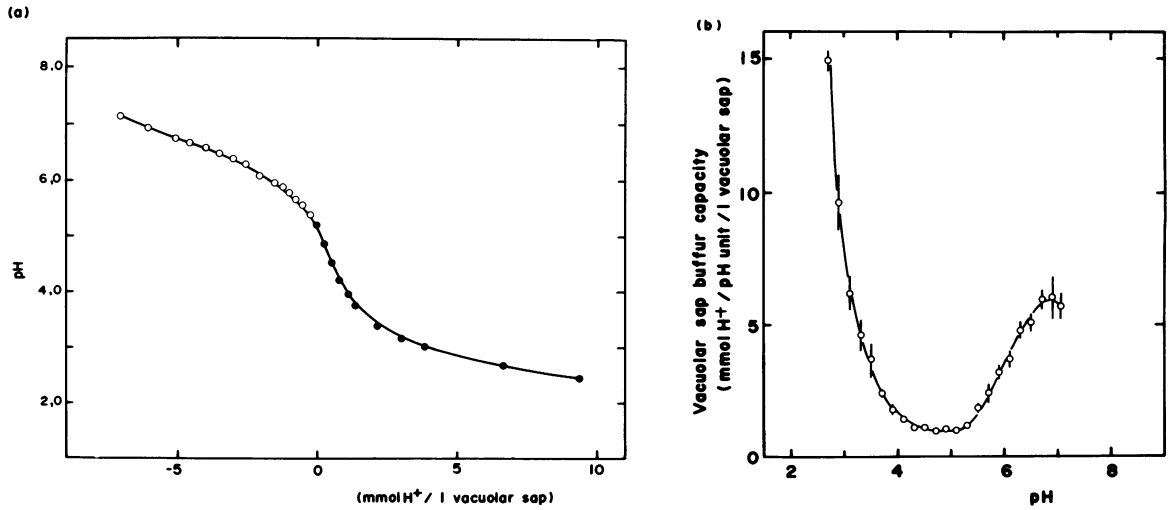

Figure 1. (a) pH titration curve of natural vacuolar sap. Open and closed circles indicate the pH titration curve of the natural vacuolar sap in the alkaline and the acidic directions, respectively. Data are representative of three independent experiments. (b) Buffer capacity of natural vacuolar sap (β_{iv}) calculated from pH titration curves. Data are shown as the mean ± standard error.

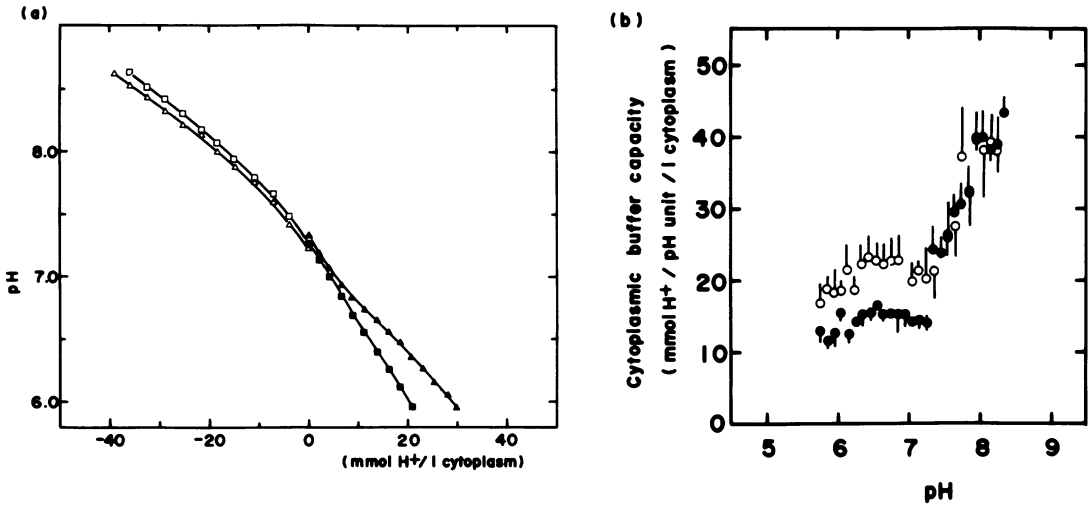

Figure 2. (a) pH titration curve of cytoplasmic solution with or without Triton X-100. Open and closed squares indicate the pH titration curve of the cytoplasmic solution without Triton X-100 in the alkaline and acidic directions, respectively. Open and closed triangles indicate the pH titration curve of the cytoplasmic solution with Triton X-100 in the alkaline and acidic directions, respectively. Each curve is the representative from six independent experiments. (b) Buffer capacity of cytoplasm (β_{ic}) with (open circles) or without (closed circles) Triton X-100 calculated from (a). Data are shown as the mean ± standard error.

volume of the cytoplasm was assumed to be 6.0% of the cell volume (14). Since the cytoplasm was mixed with a large volume of the perfusion medium, the buffer capacity of the perfusion medium was calculated and was subtracted from that of the cytoplasmic solution to obtain the intrinsic buffer capacity of the cytoplasm. The β_{ic} without Triton X-100 was 14.2 ± 0.80 mmol H^+/pH unit/L cytoplasm between pH 7.2 and 7.3. When cell organelles were permeabilized with 1% (v/v) Triton X-100, the buffer capacity in acidic region increased. This may be due to an increase of solutes having pK values of around 6.5, and may be accounted for by assuming that phosphates and bicarbonates were released from the chloroplasts by Triton X-100 treatment. Indeed, chloroplasts occupy about 10% of the cytoplasm and contain a significant amount of Pi (16.2 mM) relative to the cytosol (13.0 mM) (14). Since the stroma is an alkaline compartment (5), the pH jump observed by adding Triton X-100 may reflect the influence of the stroma. The buffer capacity of *Chara* cytoplasm was also minimal (20.35 ± 2.66 mmol H^+/pH unit/L cytoplasm) between pH 7.3 and 7.4. Sanders and Slayman (9) have also reported that β_i of the *Neurospora* cytoplasm becomes minimal at around the neutral pH. *Chara* and *Neurospora* differ from *Sinapis alba*, in which β_{ic} is maximal at around the neutral pH (1). From the curve of β_{ic} in Figure 2b, the main pH-buffering components have pK_a values which are far from 7.0. Sakano and Tazawa (8) reported that 50 to 60 mM amino acids exist in the cytoplasm of *Chara australis*. Amino and carboxyl groups of these amino acids and amino acid residues of proteins are thought to be responsible for β_{ic}.

Mechanism of pH Regulation

Present study revealed that both the cytoplasmic and the vacuolar pH values are controlled near the region where their β_i are minimal. This indicates that the plasma membrane and the tonoplast H^+ pumps can efficiently function for the pH regulation of both compartments. In Characeae the Mg·ATP-dependent H^+ extrusion from the plasma membrane of *N. obtusa* amounts to approximately 180 nmol $H^+ \cdot m^{-2} \cdot s^{-1}$ (13). If this H^+ extruding activity is imparied in a cell having a diameter of 800 μm and a relative cytoplasmic volume of 6%, the cytoplasmic pH should decrease by 1 pH unit in 1.35 s. The passive H^+ efflux from the vacuole to the cytoplasm in *C. corallina* has been estimated to be approximately 300 nmol $H^+ \cdot m^{-2} \cdot s^{-1}$ and this flux is usually balanced by an energy dependent influx of H^+ to the vacuole (4). Modifications of tonoplast H^+-pumping activities sustained by the H^+-ATPase and the H^+-pyrophosphatase (12) may well affect the pH values of both compartments. A high buffer capacity of the cytoplasm in the alkaline region may be effective to prevent the rapid alkalinization of the cytoplasm.

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