Control of Cl Influx in Chara by Cytoplasmic Cl⁻ Concentration

Dale Sanders*
Botany School, Downing Street, Cambridge CB 2 3 EA, England

Summary. Cl⁻ influx into cells of Chara corallina is shown to be stimulated by a factor of 2 to 4 by starvation of Cl⁻. The time constant for the induction of this effect is about 4.0 ksec and that for its decay when Cl⁻ is reprovided, 1.7 ksec. Intracellular perfusion of tonoplast-free cells with solutions of varying Cl⁻ concentration shows that Cl⁻ influx can be controlled directly by the concentration of Cl⁻ at the inside of the plasma membrane. Both the time course for the initial stages of induction of the starvation-stimulated flux and its absolute magnitude can be accounted for by assuming cytoplasmic Cl⁻ concentration to be the only intracellular condition to change during Cl⁻ starvation. The existence of a feedback loop between cytoplasmic Cl⁻ and Cl⁻ influx provides an alternative explanation to observations previously used in support of a Cl⁻/OH⁻ exchange hypothesis (F.A. Smith, 1972, New Phytol. 71: 595).

In fresh water Characean cells, little is known about the way in which ion transport is controlled. Most studies have attempted to elucidate the mechanism of ion transport (the energy source, cotransported ions, etc.) for given ion transport systems and have assumed that there is no tendency of the cell to counter the effects of an imposed set of experimental conditions.

In the past few years it has become increasingly recognized that the rate of ion transport across the membranes of most plant cells is controlled, not by the availability of "high energy" compounds such as ATP, which maintains a fairly constant concentration under a variety of conditions affecting ion transport (Penth & Weigl, 1969; 1971; Lilley & Hope, 1971; Miller & Spanswick, 1977), but by other factors related to cellular homeostasis. In other words, it appears that the fluxes of many ions are feedback-regulated. Thus, with respect to the fluxes of those ions which are not metabolized and whose major role in the cell is that of turgor generation or volume control, two major controlling factors are recognized (Cram, 1976): those of turgor pressure (or its equivalent for cells with extensible walls, cell volume) and of internal ion concentration.

In the fresh water Characean, Nitella, it is likely that, rather than turgor pressure or vacuolar ion concentration, internal osmotic pressure can control uptake and loss of KCl from the cell (Nakagawa, Kataoka & Tazawa, 1974), though the mechanism by which this control is exerted is unknown. However, because of the very small rate constant for turnover of ions in the Characean vacuole, such a control system would not be expected to operate quickly with respect to environmental perturbation.

The aim of the present work is to examine the nature of the controls which act on ion transport in Chara and to relate the results of this study to previous work on the mechanism of ion transport. The ion chosen for the investigation was Cl⁻. As the vacuolar ion furthest from electrochemical equilibrium with the external solution, its influx at the plasma membrane might be expected to constitute a natural control point in the regulation of net salt accumulation: in all marine algae so far investigated, turgor control is achieved by regulation of the transport of that ion whose distribution indicates it to be furthest from electrochemical equilibrium (Gutknecht & Bisson, 1977).

Materials and Methods

Cells of Chara corallina were cultured as described by Sanders (1980). Except where otherwise noted, the composition of artificial...
The final activity of K\(^{+}\) was measured using a K\(^{+}\)-specific electrode to make up the total osmolarity to 792 kPa (350 mosM).

ATP was purchased as the disodium salt from British Drug Houses Ltd., Poole, England. Free [Mg\(^{2+}\)] was calculated from the binding constants to EGTA given by Portzehl, Caldwell and Riegg (1964) and to ATP given by Wood, Davis and Lochmüller (1966). Ca\(^{2+}\) was omitted completely from the medium. Sorbitol was added to make up the total osmolarity to 792 kPa (350 mosM). The final activity of K\(^{+}\) was measured using a K\(^{+}\)-specific electrode.

**Perfusion Medium Composition**

The composition of the standard perfusion medium (PM) is given in Table 1. It is based on that shown by Williamson (1975) to be suitable for the retention of streaming in perfused cells, and of Tazawa, Kikuyama and Shimmen (1976). Tris-maleate, used by Tazawa et al. (1976) and Shimmen and Tazawa (1977) as a pH buffer was replaced by TES-KOH: Tris is known to evoke marked responses in transport phenomena in storage tissue (Van Stiphout, Mann, and Van Stiphout, 1973) and therefore lead to effects in perfused cells not present in intact cells.

ATP was purchased as the disodium salt from British Drug Houses Ltd., Poole, England. Free [Mg\(^{2+}\)] was calculated from the binding constants to EGTA given by Portzehl, Caldwell and Riegg (1964) and to ATP given by Wood, Davis and Lochmüller (1966). Ca\(^{2+}\) was omitted completely from the medium. Sorbitol was added to make up the total osmolarity to 792 kPa (350 mosM). The final activity of K\(^{+}\) was measured using a K\(^{+}\)-specific electrode.

**Intracellular Perfusion**

The aim of intracellular perfusion was the removal of the tonoplast so that direct access could be gained by the perfusing solution to the inside of the plasma membrane. The method was basically similar to that of Tazawa et al. (1976) but differed in one major respect. The method of Tazawa et al. relies on Ca\(^{2+}\)-free conditions created on the vacuolar side of the tonoplast to cause a gradual disintegration of this membrane, which occurs over 0.6 to 1.2 ksec (Van Stiphout, Mann, and Van Stiphout, 1973) and therefore lead to effects in perfused cells not present in intact cells.

ATP was purchased as the disodium salt from British Drug Houses Ltd., Poole, England. Free [Mg\(^{2+}\)] was calculated from the binding constants to EGTA given by Portzehl, Caldwell and Riegg (1964) and to ATP given by Wood, Davis and Lochmüller (1966). Ca\(^{2+}\) was omitted completely from the medium. Sorbitol was added to make up the total osmolarity to 792 kPa (350 mosM). The final activity of K\(^{+}\) was measured using a K\(^{+}\)-specific electrode.

Perfusion was continued for 100 sec. At this time the outermost of the 6 ligatures were tied. APW+250 mM sorbitol was added to the central chamber (until now, empty) and the cell regained some turgor within a few seconds. Then the middle ligatures of the two sets were tied, followed by the inner ones, which were tied just outside the central chamber. The portions of the cell between the central and outer chambers were then covered with liquid paraffin. This kept the ligatures dry, yet prevented evaporative water flow through the cell from the central chamber through the portions of cell within the innermost ligatures yet outside the central chamber. A 0.3 ksec recovery period after the end of the perfusion enabled leakage from the cell to be detected: water flow into the more concentrated interior caused disruption of the chloroplasts. If this occurred, the cell was discarded.

Observation of a portion of the cell within the central chamber was made during and after the perfusion with a Wild binocular microscope at 100 to 200× magnification. Illumination was provided by a Barr and Stroud type LS2 fiber optics light source. Both the perfusion and the subsequent experiments were performed at room temperature.

Direct measurement of the turgor pressure of perfused cells with a pressure probe (Zimmermann & Steudle, 1974) showed that they were able to maintain turgor steady at about 200 kPa for periods as long as 4 ksec. However, attempts to create a larger pressure gradient across the plasma membrane/cell wall complex were not successful: turgor always fell to 200 to 250 kPa and there remained steady. Presumably, this occurred as a result of extensive leakage of cell contents through the tied ends. For this reason, in all experiments the external solution contained 250 mM sorbitol. This restricted the turgor pressure to a level (210 to 220 kPa) where steady values could be recorded for long periods. Experiments with intact cells have shown no effect of turgor reduction to this level on the influx of Cl\(^{-}\) (Sanders, 1978).

**Cl\(^{-}\) Influx Measurements in Perfused Cells**

At the end of the recovery period, the external medium was replaced with about 1.5 ml of radioactive solution. The influx period was for 0.3 ksec. It was terminated by removing the radioactive solution and replacing with an otherwise identical solution. This