

Parallel Pathways of Potassium Transport in the Alga *Hydrodictyon reticulatum*. Effects of Calcium

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Abstract. Inflow of potassium ions into the alga *Hydrodictyon reticulatum* is reduced in the dark, the reduction being accompanied by a change in the selectivity pattern with respect to alkali metal ions, observed in competition experiments and evaluated by the gnostic analysis as described by Kovanic. This suggests that in the light a special mechanism of potassium uptake with a characteristic selectivity is switched on. This mechanism can be also suppressed by too high (2 mmol/l) or too low (EGTA) concentration of calcium ions in the medium. Since the same applies to the light-induced alkalization of the algal surroundings it seems that the light-induced potassium uptake is related to the light-induced alkalization, e.g., via exchange of external potassium cations for intracellular protons.

Key words: Potassium transport — Alga *Hydrodictyon reticulatum* — Calcium effects on potassium transport

Introduction

It is a common experience that a single ionic species may be transported across the cell membrane by several distinct parallel mechanisms. How such a plurality of parallel pathways can be recognized in practice? A complex kinetic behaviour need not always be a reliable guide. On the other hand, there seems to be a feasible assumption that the individual mechanisms (and hence also different combinations of them) might differ in their selectivity patterns towards analogues of the ion transported. This approach can be conveniently tested on the influx of potassium ions into the alga *Hydrodictyon reticulatum*. The inflow of labelled potassium into this alga is higher in the light ($1.56 \pm 0.03 \text{ pmol cm}^{-2} \text{ s}^{-1}$, $n = 45$) than in the dark ($1.06 \pm 0.09 \text{ pmol cm}^{-2} \text{ s}^{-1}$,

$n = 24$); is there just more energy available in the light for a mechanism operating already in the dark, or is there a new mechanism switched on in the light? The tonoplast of the alga lets potassium ions to pass freely from the cytoplasm into the vacuole (data to be published) and the whole cell thus behaves as a single large potassium compartment. The inflow of labelled potassium ions into a reservoir of this size remains practically linear for hours and the influx can thus be conveniently assessed both in controls and with natural analogues competing for the entry: lithium, sodium, rubidium and caesium ions. An account of preliminary data was already published (Nešpůrková et al. 1985).

Materials and Methods

Mature cells of the alga *Hydrodictyon reticulatum*, cultured in laboratory conditions as described previously (Rybová et al. 1972; Rybová et al. 1987) were used in the experiments. The basic medium for the measurements of the potassium influx contained KCl (1 mmol/l), NaCl (0.1 mmol/l), CaCl₂ (0.1 mmol/l) and ⁴²K in a concentration of the order of 10 MBq/l. The medium was modified in individual experiments by supplementation with 1 mmol/l LiCl, NaCl, KCl, RbCl or CsCl, or by manipulation with the calcium content, as described in Results. After 1000 minutes of incubation in beakers containing 100 ml of medium, the samples of algae with a wet weight (w.w.) of 20 to 40 mg were thoroughly blotted with analytical filter paper, weighed on a torsion balance and dissolved in 0.2 ml concentrated nitric acid for radioactivity measurements in a well scintillation counter. Measurements of pH in suspensions of the alga were performed with Radiometer Copenhagen PHM 62 Standard; samples of algae (250 to 300 mg) were attached by a nylon net to a combined glass-calomel electrode Radiometer KG 2320C.

The significance of the quantitative differences observed was checked by the Kovanic (1984) robust method, based on the gnostic theory of experimental data (Riemann geometry of the space of experimental values). The method does not require an a priori knowledge of the statistical distribution of the experimental data and allows to calculate the probability density. The data can then be characterized by a maximum of this function, called position parameter, and by tolerance interval (the maximum displacement of the position parameter when a new arbitrary result is added to the data). When applied to the data of the present study, the position parameters were always close to arithmetical averages, their resolution, however, was greater than it could have been achieved with the Student *t*-test (parameters of position were considered as being different whenever their tolerance intervals did not overlap).

Results

The extent of competition of different alkali metal ions with labelled potassium ions for entry to the alga *Hydrodictyon reticulatum*, as evaluated by ordinary statistics, is shown in Figs. 1 and 2 under light and dark conditions, respectively. The concentration of ⁴²K-labelled potassium as well as those of unlabelled competitors was 1 mmol/l; the entry in the absence of competitors corresponds

to the control value (100%). Some of the differences between the inhibitory patterns in the light and in the dark are clearly expressed. In the light it is lithium which has the strongest inhibitory effect on the potassium entry, whereas sodium has the least influence. In the dark it is rubidium or caesium which compete most effectively with potassium ions for the entry, unlabelled potassium ions having a lower effect, and both sodium and lithium having little influence. The following sequences express the competitive power of individual alkali metal ionic species when the arithmetic averages are considered as exact figures:

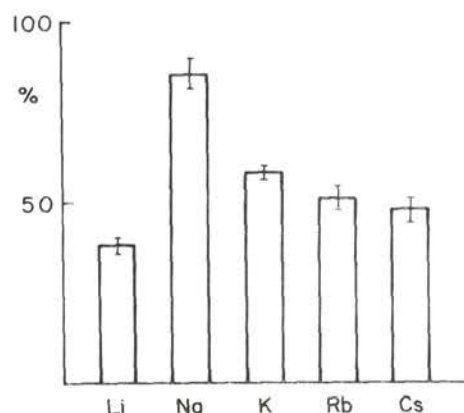


Fig. 1. Inflow of $^{42}\text{K}^+$ to algal cells in per cent of control values after 1000 min of incubation in the light and in the presence of Li^+ ($n = 9$), Na^+ ($n = 9$), K^+ ($n = 15$), Rb^+ ($n = 15$) or Cs^+ ($n = 15$) chloride.

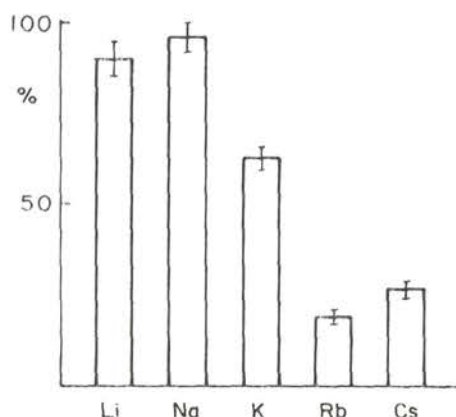


Fig. 2. Inflow of $^{42}\text{K}^+$ to algal cells in per cent of control values after 1000 min of incubation in the dark and in the presence of Li^+ ($n = 9$), Na^+ ($n = 9$), K^+ ($n = 14$), Rb^+ ($n = 14$) or Cs^+ ($n = 14$) chloride.

$\text{Li}^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+$ in the light,
and $\text{Rb}^+ > \text{Cs}^+ > \text{K}^+ > \text{Li}^+ > \text{Na}^+$ in the dark.

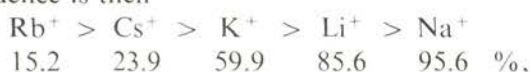
However, it is obvious that it is too bold to decide about the succession of $\text{Cs}^+ > \text{Rb}^+ > \text{K}^+$ in the light and $\text{Li}^+ > \text{Na}^+$ or $\text{Rb}^+ > \text{Cs}^+$ in the dark on the basis of ordinary statistics. For this reason, a more detailed analysis of the data was carried out by the gnostic method of Kovanic (1984). In this way the following light sequence was obtained

$\text{Li}^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+$
36.5 43.4 50.3 57.5 80.6 %,

where the percentages correspond to the "position parameters", the gnostic analogues of the arithmetic averages, i.e. the levels to which the inflow of labelled

potassium is suppressed by competition. The corresponding tolerance intervals are 35.4–37.7 for Li^+ , 42.3–44.7 for Cs^+ , 49.3–51.4 for Rb^+ , 56.7–58.3 for K^+ and 78.5–83.4 for Na^+ .

The dark sequence is then



with tolerance intervals 14.8–15.7 for Rb^+ , 22.8–25.7 for Cs^+ , 58.2–62.0 for K^+ , 82.4–90.3 for Li^+ and 91.9–107.0 for Na^+ .

It can be seen that the gnostic method gives the same sequences as does the simple succession of arithmetic averages; however, the sensitivity, or resolution power of the former, is superior to that of ordinary statistics. In Figures 3 and 4, the probability density of the gnostic method is plotted in arbitrary units, and the probability maxima correspond to the position parameters, shown below together with the tolerance intervals. The gnostic method is used throughout the rest of the present paper to evaluate the selectivity patterns of the potassium entry to the alga *Hydrodictyon reticulatum* under various experimental conditions.

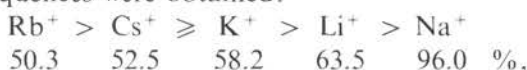
In the following, the selectivity patterns of the potassium entry were examined in three media, differing in their calcium levels:

Medium I: CaCl_2 2 mmol/l, KCl 1 mmol/l, NaCl 0.1 mmol/l;

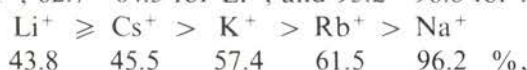
Medium II: KCl 1 mmol/l, NaCl 0.1 mmol/l;

Medium III: EGTA $7.5 \cdot 10^{-6}$ mol/l, KCl 1 mmol/l, NaCl 0.1 mmol/l.

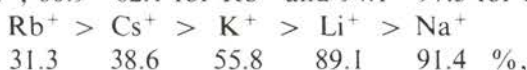
The following sequences were obtained:



for medium I, with tolerance intervals 49.5–51.0 for Rb^+ , 50.8–53.9 for Cs^+ , 57.6–58.8 for K^+ , 62.7–64.3 for Li^+ , and 95.2–96.8 for Na^+ ;



for medium II, with tolerance intervals 43.1–46.2 for Li^+ , 44.8–46.3 for Cs^+ , 56.4–58.4 for K^+ , 60.9–62.1 for Rb^+ and 94.1–97.3 for Na^+ , and, finally



for medium III, with the following gnostic tolerance intervals: 30.1–32.6 for Rb^+ , 37.5–52.6 for Cs^+ , 55.1–56.6 for K^+ , 87.3–90.8 for Li^+ and 88.9–93.6 for Na^+ .

It can be noted that the sequence found for medium II (with nominally zero, in fact low, calcium concentration) is very similar to that found formerly for control conditions in the light, with only Rb^+ and K^+ having their position interchanged, whereas those obtained with high (2 mmol/l) calcium concentration as well as with very low calcium concentration in the presence of EGTA

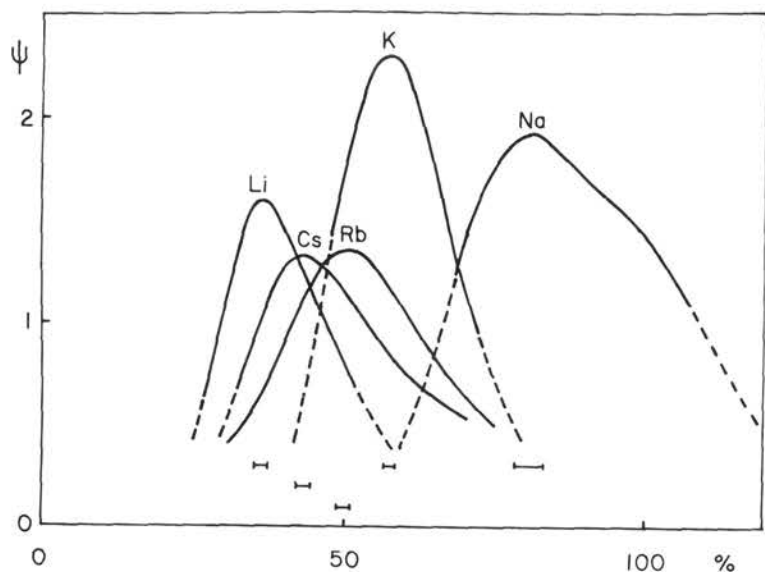


Fig. 3. The relationship of probability density ψ (in arbitrary units) for competing alkali metal ions in the light and $^{42}\text{K}^+$ (in per cent of controls) in the cells of *Hydrodictyon reticulatum*. Solid bars represent the tolerance intervals for the gnostic analysis.

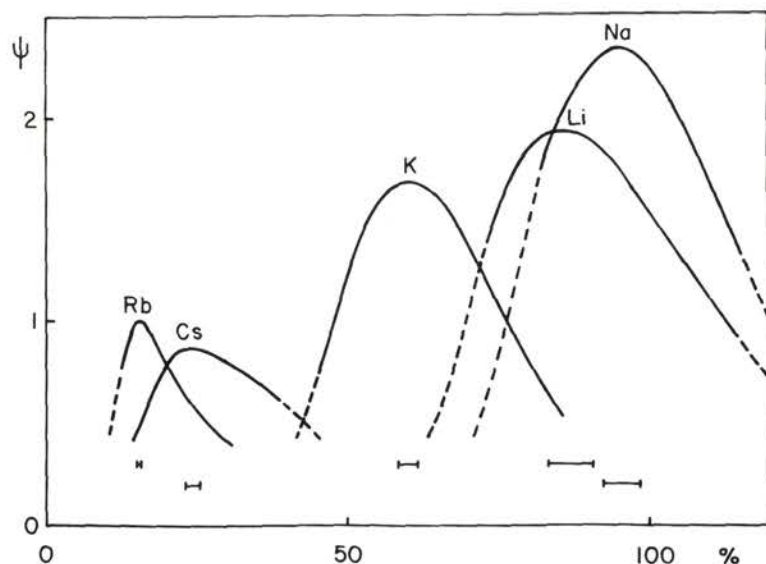


Fig. 4. The relationship of probability density ψ (in arbitrary units) for competing alkali metal ions in the dark and $^{42}\text{K}^+$ (in per cent of controls) in the cells of *Hydrodictyon reticulatum*. Solid bars represent the tolerance intervals for the gnostic analysis.

(ethyleneglycol-bis(β -aminoethylether)N,N'-tetraacetic acid, a chelating agent for calcium ions) correspond to the sequence obtained in the dark. In media I and III the inflow of potassium ions is decreased as compared with the medium containing 0.1 mmol/l Ca^{2+} (on average by about 30 % in the former, and by about 5 % in the latter), in medium II the control inflow remains unchanged.

However, the medium with high calcium (medium I) and the medium with EGTA (medium III) are similar also in another respect; they reduce the ability of the alga *Hydrodictyon reticulatum* to alkalinize its surroundings in the light. The measurements can be easily performed with thick suspensions of the algae attached to glass pH-electrodes by nylon nets (Rybová et al. 1980). From Figures 5 and 6 it is obvious that both high calcium and EGTA interfere with the alkalinizing ability of the algal cells.

Discussion

In the dark, the inflow of potassium ions to the alga *Hydrodictyon reticulatum* is reduced and at the same time its characteristic selectivity pattern $\text{Li}^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+$ is changed into $\text{Rb}^+ > \text{Cs}^+ > \text{K}^+ > \text{Li}^+ > \text{Na}^+$.

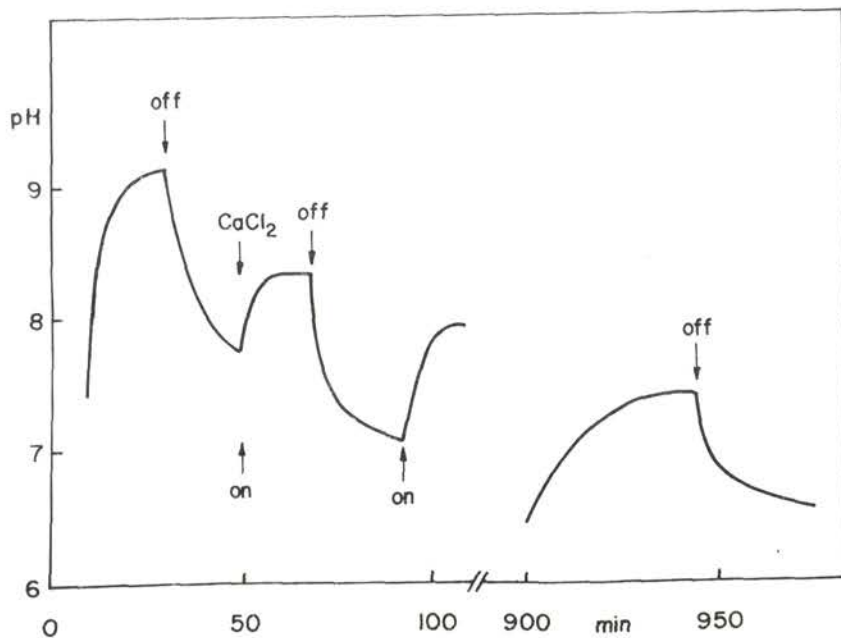


Fig. 5. Effects of 2 mmol/l CaCl_2 on the light and dark-induced changes in pH in the suspension medium of the alga *Hydrodictyon reticulatum*.

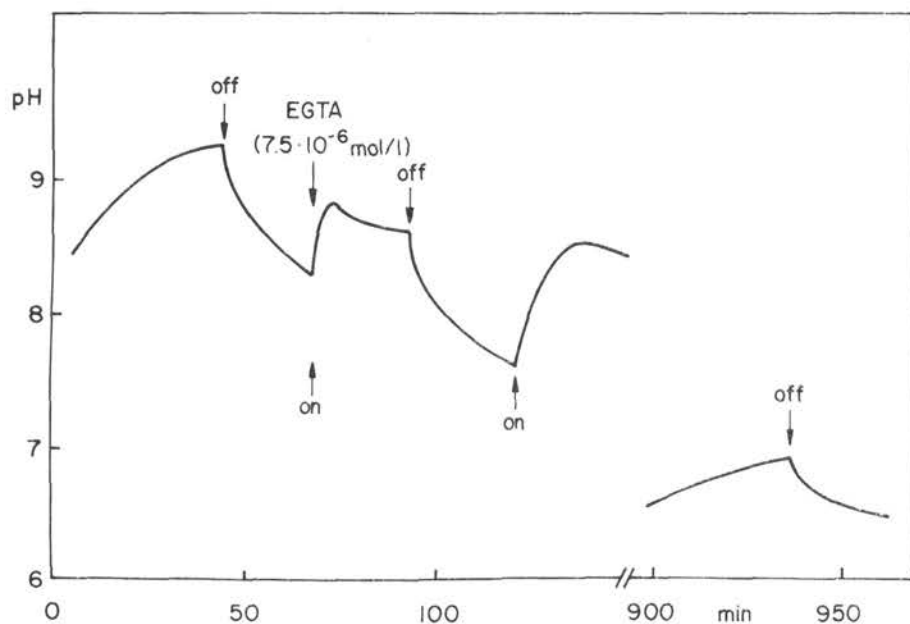


Fig. 6. Effect of EGTA (added to medium with no Ca^{2+}) on the light and dark-induced changes in pH in the suspension medium of the alga *Hydrodictyon reticulatum*.

This observation suggests that the higher uptake of potassium ions in illuminated cells is not a simple result of more energy available, but rather that a new transport mechanism, with a profoundly different selectivity pattern (preferentially lithium selective), is switched on by the light.

In media containing either 2 mmol/l calcium ions or EGTA the behaviour of the alga with respect to potassium uptake is similar to that in darkness. Alkalinization of the medium in the light, which is a process reflecting the uptake of bicarbonate (Rybová et al. 1980), is also suppressed under these two conditions, as it is in darkness. Hence it may be speculated that the mechanism of potassium uptake operating only in the light is somehow related to the process of alkalinization and represents, e.g., exchange of potassium ions for protons.

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