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ION AND WATER TRANSPORT IN LIMONIUM

V. THE IONIC STATUS OF CHLOROPLASTS IN THE LEAF OF *LIMONIUM VULGARE* IN RELATION TO THE ACTIVITY OF THE SALT GLANDS

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SUMMARY

A freeze-drying and non-aqueous extraction technique has been used to record the salt content of chloroplasts in leaf segments of *Limonium vulgare*. Changes in the level of K^+, Na^+ and Cl^- were observed when leaf segments were treated with NaCl or KCl solutions which induce pumping of the salt glands. The effects of treatments designed to inhibit glandular activity are also recorded.

Net accumulation of ions into chloroplasts is shown to be a process *in vivo*. The results provide evidence for a direct effect of glandular activity on cytoplasmic ion levels and they provide intracellular evidence for the induction of glandular activity after a certain lag period.

INTRODUCTION

There is good evidence that the gland-cell complex of the leaves of Limonium species constitutes a mechanism for lowering ionic activities in the photosynthetic parenchyma[1-5]. However, the complex compartmentation of salts in plant cells in general and the difficulty of studying the problem in this tissue, in particular, makes the proof of this statement difficult. It is interesting from the point of view of the physiology and ecology of these plants to know which of the compartments is most affected by the pumping mechanism of the gland-cell complex and what is the critical concentration in this compartment at which the pumping mechanism is triggered.

It has been possible to analyse the kinetics of ion transport through the gland complex, and this analysis indicates direct connection with the leaf cytoplasmic compartment[13]. An experimental test of this would be to show that the ionic content of a cytoplasmic organelle responds directly to glandular activity. Recently it has been shown that chloroplasts constitute a compartment of high salt concentration in green cells[6]. Kinetic analysis in the giant algal cell of *Tolypella intricata* with

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radioactive Cl^- has indicated that the chloroplasts exchange Cl^- with a compartment of the cytoplasm[7]. This exchange was much faster than the exchange of Cl^- between the vacuole and a compartment of the cytoplasm. The chloroplast compartment therefore reflects changes in the labelling of the cytoplasm. However, in these experiments conditions of flux equilibrium were maintained as far as possible and no net change in the ion concentration of any compartment was found. In the leaf tissue of *Limonium vulgare* under conditions such as are found during treatment of leaf discs of low salt status with high external salt concentrations full secretory activity of the salt glands is reached only after a period of 3–4 h (ref. 8), during which time it can be assumed that the ionic activities in the various compartments of the parenchyma cells are undergoing changes.

Changes in the levels of K^+, Na^+, and Cl^- were indeed found in the chloroplasts during the early stages of salt treatment. These results together with some further data on tissue where the salt glands have been partially inhibited provide evidence on the important question of the ionic relations of this system.

METHODS

L. vulgare Mill collected from an East Anglian saltmarsh was grown on soil watered with tap water. The salt status of the plants was therefore low and leaf discs taken straight from the plant showed no secretion unless pretreated with a salt solution. Pretreatment was effected by floating 50 g fresh weight per treatment of freshly cut leaf segments onto aerated salt solutions, usually 100 mM KCl or NaCl. The treatments were kept under conditions of constant illumination during each experiment at a temperature of 25°.

Sampling of material during the time-course of each experiment was effected by rapidly freezing in liquid N_2. Chloroplasts were extracted from freeze-dried tissue in the manner described previously using a non-aqueous technique[6].

Analysis of the chloroplast material was carried out on fresh extracts stored in a vacuum dessicator in darkness. Cl^- was measured by an electrometric method[9], K^+ and Na^+ were estimated by flame photometry. Freezing point depressions were measured using the technique and apparatus of RAMSAY AND BROWN[10]. Chlorophyll was estimated by the method of MACKINNEY[11], and the packed volume of aqueously isolated chloroplasts was found using the technique previously described[6].

RESULTS

Treatment of leaf segments of low salt status with a solution of 100 mM NaCl (Expt. 1, Fig. 1) resulted in a rise in the NaCl content of the chloroplasts from 0.19 to 0.61 μmole/mg dry weight (Fig. 1), *i.e.* an increase of over 300%. The initial content of K^+ was high, comparatively, and this level was maintained throughout except for a slight reduction over the 1st h. However, after 3 h a reversal in the rise in NaCl content is evident. It is at approx. 3 h that the salt glands reach their full secretory capacity in NaCl solution and the marked changes in NaCl content around this time period are taken to be a reflection of this activity on the ion content of the parenchymal cytoplasm.

Since it is apparent from previous work[6] that the level of K^+ in chloroplasts

is controlled to a greater extent than Na^+ it was of great interest to carry out another experiment using 100 mM KCl in place of NaCl (Expt. 2). The results are shown in Fig. 2. It is evident that the K^+ level does not vary to such a great extent as the Na^+ level in the previous experiment. Nevertheless an inflexion in the curve at 4 h is again taken as a reflexion of the induction of full activity of the salt glands. It is interesting that the Na^+ level, initially quite low, does not decrease to any great extent, being reduced to about 70 % of its previous level; this level is maintained for 6 h despite the changes in the K^+ level and the activity of the salt glands.

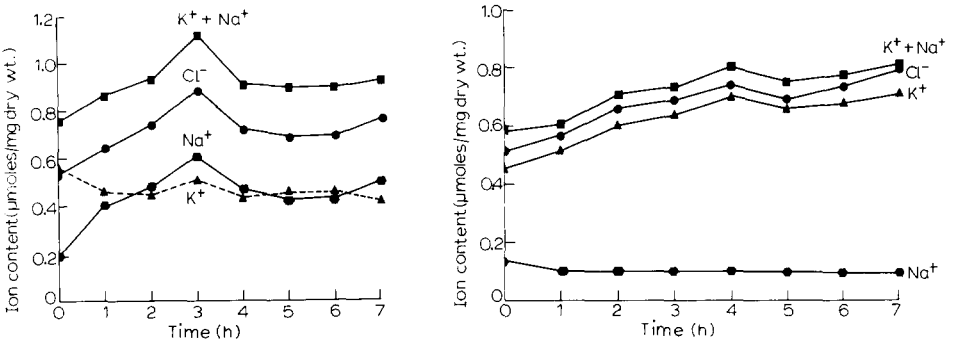

Fig. 1. Changes in the Na^+, K^+ and Cl^- content of chloroplasts in leaf segments of *L. vulgare* during incubation on a solution of 100 mM NaCl. The experimental points are the average values from two replicates run simultaneously. The segments were kept under constant illumination at a temperature of 25°.

Fig. 2. Changes in the Na^+, K^+ and Cl^- content of chloroplasts in leaf segments of *L. vulgare* during incubation on a solution of 100 mM KCl. Experimental details as for Fig. 1.

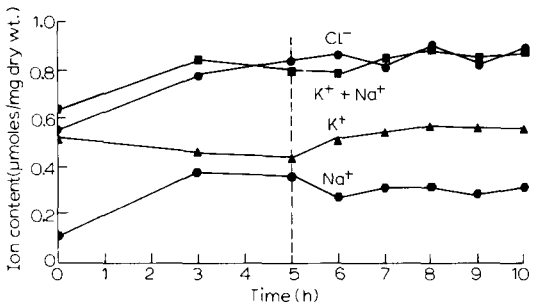

Fig. 3. Changes in the Na^+, K^+ and Cl^- content of chloroplasts in leaf segments of *L. vulgare* during incubation on a solution of 50 mM NaCl + 50 mM KCl. Experimental details as for Fig. 1.

In a further experiment (Expt. 3, Fig. 3) the bathing solution of 100 mM NaCl was changed at 5 h for a solution of 50 mM NaCl + 50 mM KCl. The initial changes are similar to those found in the first experiment (Fig. 1), but the effects of the changeover of composition of the bathing solution at 5 h indicate that a period of cation readjustment takes place in the chloroplast, similar to that of the cytoplasm.

It is possible to inhibit the electrogenic component of the glandular Cl^- efflux by incorporating benzenesulphonate ions into the bathing medium. In Table I the results of two experiments (Expts. 4 and 5) are shown in which the composition of the medium was 100 mM NaCl, 100 mM choline benzenesulphonate. The data

TABLE I

THE EFFECT OF BATHING SOLUTIONS WHICH STIMULATE OR INHIBIT SALT-GLAND PUMPING ON THE SALT CONTENT OF CHLOROPLASTS IN LEAF SEGMENTS OF *L. vulgare*

The values are the average of two replicate treatments.

	Chloroplast ion content (μmoles/mg dry wt.)			
	K^+	Na^+	$K^+ + Na^+$	Cl^-
Expt. 4				
Control, 6 h in 100 mM NaCl (salt glands pumping)	0.54	0.46	1.00	0.75
Salt glands inhibited, 6 h in 100 mM NaCl + 100 mM choline benzenesulphonate	0.54	0.96	1.50	1.25
Expt. 5				
Untreated (salt glands not pumping)	0.71	0.38	1.08	0.87
Salt gland pumping, 24 h in 100 mM NaCl	0.53	0.29	0.82	1.45
Salt glands inhibited, 24 h in 100 mM NaCl + 100 mM choline benzenesulphonate	0.56	0.63	1.19	0.99

show that in both experiments the inhibition causes high levels of Na^+ in the chloroplasts while the K^+ level is maintained constant. In Expt. 4 the difference in levels of Na^+ (0.50 μmole/mg dry weight) is matched by the difference in Cl^- (0.50 μmole). The charge imbalance between the $K^+ + Na^+$ level and the Cl^- level (0.25 μmole less anion) is probably the result partly of fixed negative charge groups and partly of other, unidentified anions. It is evident from the previous experiments that the imbalance between $K^+ + Na^+$ and Cl^- varies between experiments and sometimes during an experiment. It is possible that organic anions play a role in maintaining charge neutrality. In Expt. 5 the low Na^+ and Cl^- contents after 24 h indicate that Cl^- and benzenesulphonate ions may be replacing other ions in the chloroplast.

The packed volume of chloroplasts isolated in a buffered sorbitol solution[15] was found to be 101.1 ± 4.8 (S.E., $n = 6$) μl/mg chlorophyll (corrected for 26% interspace volume of the packed chloroplasts). The non-aqueously isolated chloroplasts weighed 35.3 ± 0.6 (S. E., $n = 6$) mg/mg chlorophyll (corrected for an estimated 10% loss of chlorophyll during the extraction procedure). Therefore unit dry weight of chloroplasts (mg), the basis on which the ion contents have been expressed, is equivalent to a volume of 2.86 μl of aqueously isolated chloroplasts. Of this volume, up to 1 μl will represent the contribution of structural, non-osmotic material (assuming an overall specific gravity of this material of unity). It is therefore probable that the osmotic space of the chloroplasts is about 2 μl/mg dry weight. On this basis the range of concentrations in the chloroplasts encountered in the previous experiments were: K^+, 200–350 mM; Na^+, 50–500 mM; Cl^-, 250–700 mM. Freezing-point-depression determinations on chloroplast material (NaCl treatment of Expt. 4, Table I) gave a value of 120 mM for 1 mg dissolved in 10 μl water. This represents for the intact chloroplast a total concentration of all solutions of 600 mM, which is only 100 mM greater than the mean estimated $K^+ + Na^+$ concentration of this treatment.

This difference may be made up by Ca^{2+} and Mg^{2+} determined previously[6]. These five ions must constitute the major part of the osmoticum of the chloroplasts in these experiments.

DISCUSSION

The observation of numerous plasmodesmata-connecting gland cells to the cytoplasm of the neighbouring chlorenchyma[12], and the kinetic analysis of transit fluxes[13] in Limonium indicate that the gland cells directly control the cytoplasmic content in this tissue. The work on chloroplasts of giant algal cells[7] indicates that at least part of the cytoplasmic Cl^- exchanges fairly rapidly with part or all of the chloroplast Cl^-. If the same relationship holds for K^+ and Na^+, then the chloroplast ion content would reflect quite rapidly changes in the cytoplasmic content which in turn would reflect the pumping activity of the salt glands.

In an analysis of the ionic spaces in Limonium there appears a Cl^- space intermediate between cytoplasm and vacuole with a half-time of approx. 130 min; such an extra space is not observed however for Na^+ (ref. 13). If this space represents the chloroplast compartment then the result would be in agreement with the observations of SALTMAN *et al.*[14] who found that the chloroplasts of *Nitella opaca* have an efflux half-time of approx. 100 min for halide ion and 20 min for Na^+. A Na^+ half-time of this magnitude would be undetectable in Limonium as it differs little from that of the cytoplasm. There are indications that the ions of the chloroplast are not altogether freely exchangeable, however. The results on Cl^- exchange in the giant algal cell of *Tolypella intricata* tentatively point to an ion compartment within the chloroplast which is only very slowly exchangeable with the cytoplasm. In Expts. 1 and 2 (Figs. 1 and 2, respectively) the rise of the total salt level in the chloroplast is arrested after 3–4 h and is subsequently held at a steady level. In Expt. 1 the K^+ declines from its initial level to give a steady value, and likewise Na^+ in Expt. 2. We interpret these results in the following way: when presented with a salt load the cytoplasm fills to a level determined by the plasmalemma pumps, with a half-time of about 20 min[13]; the chloroplasts however continue to accumulate NaCl in a linear fashion for at least 3 h, and this indicates that there are inwardly-directed ion pumps at a chloroplast surface. The slow rate of filling is not inconsistent with the long time constant of the intermediate Cl^- space[13], which would suggest Cl^- pumping to be rate controlling. From about 100 min the glandular transport mechanisms become effective, rising to maximum values throughout another 100 min[8], and bringing the cytoplasmic concentration to a new lower steady state. The longer time constant of the chloroplast phase then causes an overshoot in chloroplast ion content, because for a time the chloroplasts will still be accumulating ions whilst the cytoplasmic content is falling. The slight rises about the 7th h are probably due to a fall off in gland pumping rate, which is often observed as a slight fall in short-circuit current in a voltage clamp experiment. The slight fall in endogenous K^+ in Expt. 1 and Na^+ in Expt. 2 must be due in part to their replacement by the complementary ion, and their steady levels thereafter to the buffering effect of the vacuolar compartment. Expts. 1 and 2 also indicate that treatment of tissue with NaCl does not appear to induce K^+ transport, and *vice versa*.

In Expt. 3 (Fig. 3) the medium changeover at 5 h to 50 mM NaCl + 50 mM

KCl solution causes a fall in chloroplast Na^+ and a rise in K^+, as one might expect; the Cl^- content however remains approximately constant at its steady state value, which again suggests control by a Cl^- pump.

The calculated ionic concentrations in the chloroplast are quite high and it might well be supposed that the glandular pumps are not really controlling the level of chloroplast salt; if the chloroplast uptake mechanism is constitutive however, any appreciable rise in the cytoplasmic concentration would lead to prohibitively high chloroplast ion activities. It can be seen from Table I that partial inhibition of glandular Cl^- transport does in fact lead to much higher levels of Na^+ and Cl^- in the chloroplast.

To summarise we would say that the datum here indicates direct changes in the cytoplasmic ion levels due to glandular transport; that it shows net accumulation into chloroplasts as a process *in vivo*; and that it provides intracellular evidence of the induction of glandular transport after a certain lag period.

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