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ION AND WATER TRANSPORT IN LIMONIUM

IV. DELAY EFFECTS IN THE TRANSPORT PROCESS

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SUMMARY

The initiation of ion pumping has been studied in Limonium leaf discs of low-salt status. The parameters of glandular transport, *i.e.*, short-circuit current, potential difference, volume efflux, and the fluxes of $^{22}Na^+$ and $^{36}Cl^-$, all show a sigmoid rise to steady-state values, with pronounced but similar lag periods. It is proposed that the form of the rise is incompatible with any diffusion controlled process and is due to the induction of ion pumps.

INTRODUCTION

When Limonium is grown on a salt-free medium for some time it loses the ability to actively transport ions with its leaf gland cells when supplied with salt. This ability is regained after a period of 3–4 h in NaCl solution, during which time the various parameters associated with the active ion transport increase until they assume values shown by leaf discs used in previous experiments[1], which were pretreated with salt solutions for many hours beforehand. In this paper the delay period is described in detail for the electrical parameters short-circuit current and transglandular potential difference, for the radioisotope fluxes of Na^+ and Cl^-, and for the volume efflux J_V.

The cells of the Limonium salt gland are connected to the surrounding cells by numerous plasmodesmata, and it is through these that the ions which are extruded by the gland cells must move.

The movement of ions to the gland through the intercellular spaces could provide an alternative pathway, but this is highly unlikely in view of the extensive internal cuticularisation of the gland cell complex. However, the effect of the diffusion path to the active transport site in contributing to the delay period has been separately determined, and this analysis is presented in another paper[2].

METHODS

Limonium vulgare was collected from submaritime habitats and cultured on soil with occasional doses of 5 times diluted Hoagland solution. The short-circuit

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technique for Limonium leaf preparations has been described previously[1]. In these studies the same general circuit was used together with an additional electrometer for measuring the accuracy of the voltage clamping; during measurement of the short-circuit current the potential could be held to an accuracy of $\pm$ 0.5 mV. *In vivo* the glands transport ions and water out of the leaf and, in these experiments, to the outer chamber; all potential differences are referred to the inner chamber as zero. During the voltage clamp the radioisotope fluxes of $^{22}Na^+$ and $^{36}Cl^-$ were measured over the preparation; the outer chamber was connected by polythene tubing to a scintillation flow-cell containing an organic scintillator in perspex. This was connected to a photomultiplier and seated in a lead castle and was monitored with an automatic scalar–timer with print-out facility. A peristaltic flow inducer was used to circulate the solution in this closed system, the total volume being approx. 4 ml. The circulation time of this volume was set at 5 sec, and as the counts were summed over 300-sec intervals, the resolution of the system was more than adequate. Turbulence in the outer chamber and in the flow cell caused good mixing, although the peristalsis gave rise to small oscillating streaming potentials, presumably at the tips of the salt bridges, requiring a circuit with slower rise time to lose them. The set-up was calibrated by injecting standard radioisotope samples into the flow system, and was found to be linear at all concentrations used. Fresh leaf preparations of plants of very low salt status were mounted directly between the half-chambers in a solution of 100 mM $^{22}NaCl$ or $Na^{36}Cl$ and short-circuit current, potential difference or ion fluxes were measured from time zero. The volume efflux measurements were described previously[3], and were again carried out by recording the volume of solution emerging from a gland as a function of time, under a layer of *n*-decane; the leaf preparation rested on a pad saturated with 100 mM NaCl solution, in a saturated atmosphere.

RESULTS

The time-course of the change in potential difference across the preparation, and also of the short-circuit current, can be described by a displaced sigmoidal relationship extending over several hours (Figs. 1 and 2). Occasionally there is an initial fast transient lasting a few minutes which can be regarded as a diffusion current, which is revealed by voltage clamp. There is then a period of about 2 h within which both the potential difference and current are very close to zero; they rise to assume relatively constant values, over a period of approximately another 2 h.

The rise of radioactivity in the scintillation flow system followed a similar pattern, when leaf discs were mounted with 100 mM $^{22}NaCl$ or $Na^{36}Cl$, and clamped at 0 (Fig. 3) and at 100 mV (Fig. 4). In experiments where a non-zero voltage clamp was used, the lag period was greater and more clearly defined. In efflux experiments with $^{22}Na^+$ the outer chamber was clamped at +100 mV and at −100 mV during $^{36}Cl^-$ fluxes; both clamps therefore reduced the passive component of the total ion transfer. In Fig. 5 is shown a backflux experiment in which the $^{22}Na^+$ and $^{36}Cl^-$ were held in the outer chamber and the appearance of radioactivity in the inner chamber was recorded with the flow system; the backfluxes of both ions show no real lag periods, and steady rates are established quite quickly.

Volume flows as a function of time show the same pattern, but as the transfer across individual glands can be followed under the microscope, the system is capable

of greater resolution. In several experiments the efflux J_v of four neighbouring glands was studied when their supporting tissue was bathed with 100 mM NaCl solution (Fig. 6). The four salt glands show different lag periods, culminating in different rates of exudation in the steady state.

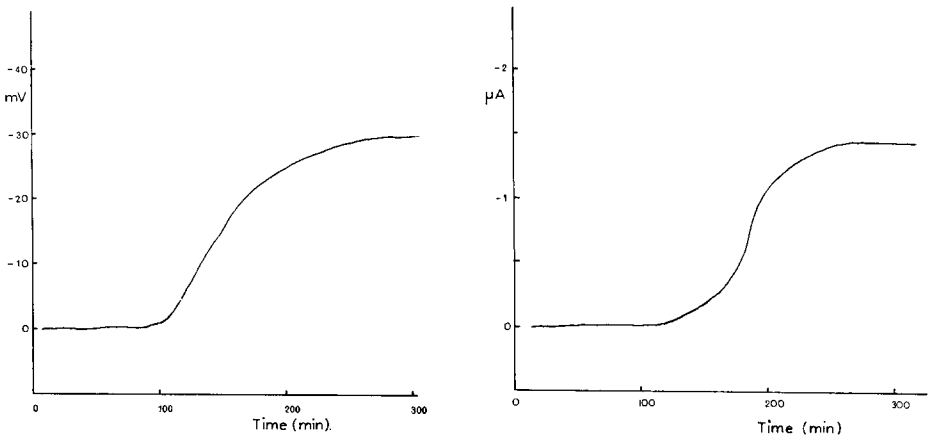

Fig. 1. Rise of potential across a fresh preparation when treated with 100 mM NaCl. The curve is a recorder tracing for a representative disc.

Fig. 2. Rise of short-circuit current across a fresh preparation when treated with 100 mM NaCl. The curve is a recorder tracing for a representative disc.

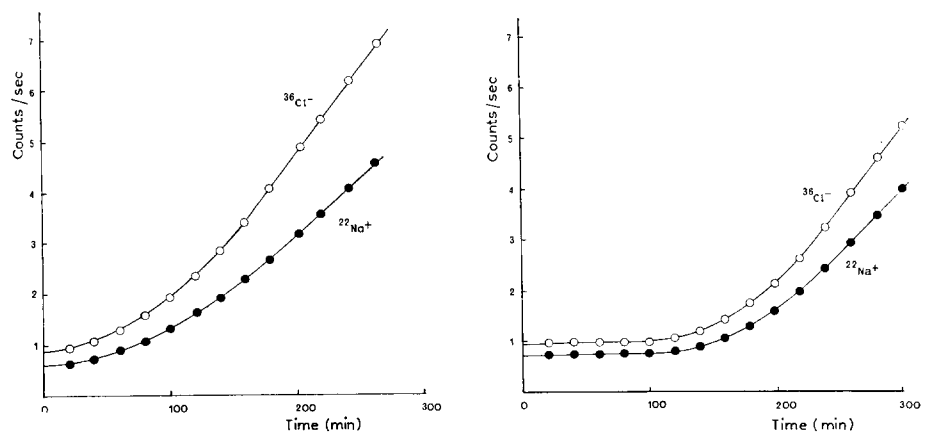

Fig. 3. The transfer of Na^+ and Cl^- across a fresh preparation, short circuit at 0 mV. The ordinate represents the activity of the outer chamber, in a representative experiment.

Fig. 4. The transfer of Na^+ and Cl^- across a fresh preparation with outer chamber clamped at $\pm$ 100 mV. The ordinate represents the activity of the outer chamber in a representative experiment. The clamp is negative for Cl^- and positive for Na^+.

The effect of transferring the disc from one concentration to another is illustrated in a single experiment (Fig. 7); here the short-circuit current is the measured parameter as a function of time. A disc was treated with 100 mM NaCl and the current rose sigmoidally to a steady value. The chambers were then filled with 200 mM NaCl

and the current rose sigmoidally to a higher value, with a lag period of similar duration. When the concentration was reduced to 100 mM the current slowly decayed to its former value.

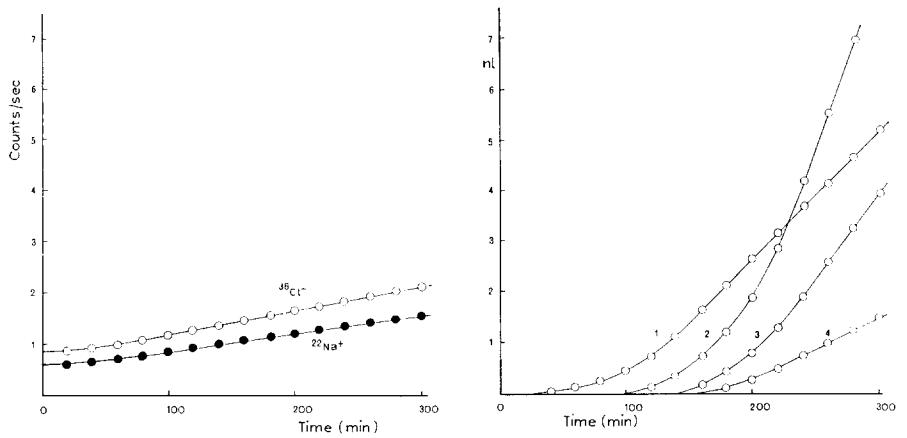

Fig. 5. Backfluxes of Na^+ and Cl^- across a fresh preparation, short circuit at 0 mV. The ordinate represents the activity in the inner chamber, in a representative experiment.

Fig. 6. Volume efflux from four nearest neighbouring glands on the surface. The ordinate represents the volume of solution leaving a single gland, during a representative experiment.

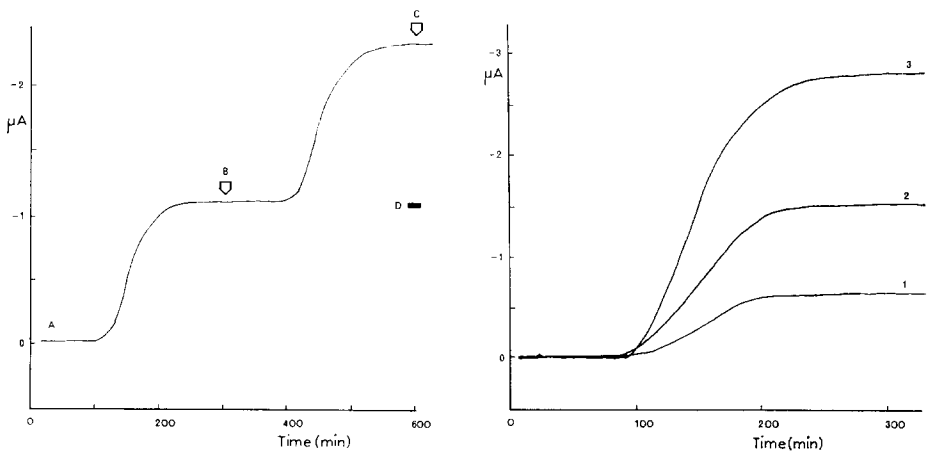

Fig. 7. Short-circuit current changes over a representative short-circuited disc during changes in salt concentration. A, fresh disc bathed in 100 mM NaCl from zero time; B, bathing medium changed to 200 mM NaCl; C, bathing medium returned to 100 mM NaCl; D, mean level of short-circuit current 12 h after C.

Fig. 8. Rise of short-circuit current in three fresh discs from one leaf blade. 1, bathed from time zero in 50 mM NaCl; 2, in 100 mM NaCl; 3, in 200 mM NaCl.

The effect of concentration on the lag period was also investigated, and Fig. 8 shows the results of a typical experiment; the measured parameter was again short-circuit current. Three discs were removed from one leaf blade and mounted, one after the other, with solutions of 50, 100 and 200 mM NaCl. The final short-circuit currents were of increasing size, but the lag period was remarkably constant.

In a final experiment, a few plants were transferred to aqueous culture and grown hydroponically in aerated artificial seawater for several weeks. This medium had a total Cl^- molarity of 0.56; the plants were actively pumping salt and crystals covered the leaves. When leaf discs were mounted in 100 mM NaCl solution and voltage clamped at zero, the short-circuit current had an initial steady value between -0.5 and -1.0 μA, and after a lag period of duration comparable to that in other experiments it rose sigmoidally to a higher value (Fig. 9), a value greater than that to be expected from previous studies at this concentration, however.

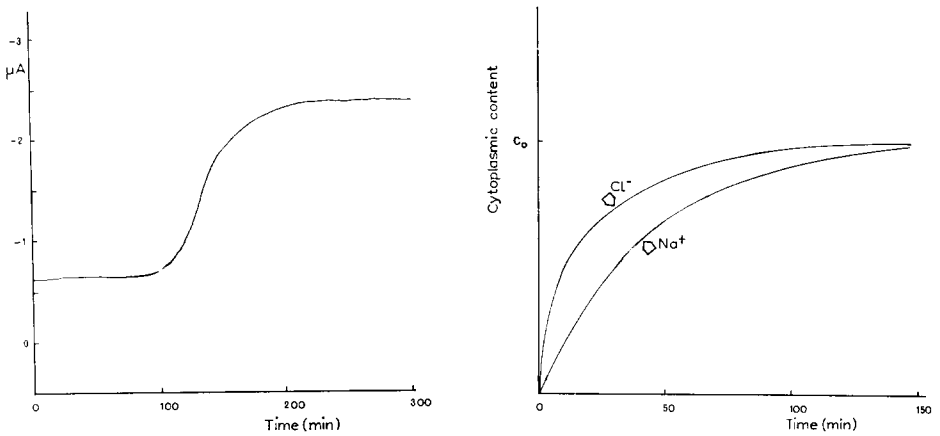

Fig. 9. Rise of short-circuit current in a sample disc bathed in 100 mM NaCl from a plant grown on seawater.

Fig. 10. The rise of NaCl concentration in the cytoplasm during a step load. The bounding values are for the individual ions, based on the half-times obtained from washouts.

DISCUSSION

In a companion paper[2] a study is made of the different ionic compartments in Limonium tissue, and by comparing the time constants of these with the time constant for isotopic transfer across the preparation by the gland cells, it can be shown that the glands are transferring ions directly from the second slowest compartment, assigned to the cytoplasm. This assignment is, however, not of direct importance to any kinetic argument; it therefore follows that when Limonium tissue of low-salt status is given a step function change in external salt concentration, the cytoplasmic salt concentration should rise asymptotically with a half-time lying somewhere between the half-times of Cl^- (approx. 10 min) and Na^+ (approx. 30 min), as shown in Fig. 10. If the salt glands possess constitutive transfer mechanisms and if they sample directly from the cytoplasm then the various parameters of transport, *e.g.*, short-circuit current, should show rises similar both in time and form; this is not so, however. An obvious explanation for this behaviour is that in the cytoplasm–gland system there exists a process which itself generates a sigmoid relationship of activity *versus* concentration; an allosteric ion pump would be an example.

The filling of the cytoplasm is given by the equation

$$c = c_0(1 - e^{-kt})$$

where c_0 is the steady-state concentration, and k is the cytoplasm rate constant. When the short-circuit current is measured from zero time in solution of different concentration it can be seen that as the medium concentration is raised, any particular cytoplasmic concentration is reached at progressively shorter times. The lag period should therefore shorten and the current rise sooner, the higher the external concentration. As it can be seen from Fig. 8, this is not the case, and thus the general shape of the curve cannot be explained by any such simple physical process.

The numerous symplastic connections between leaf cells and the plasmatic connections of the gland to the neighbouring cells should act to equalise their time constants; four neighbouring glands should therefore show very similar behaviour. The volume efflux *versus* time curves shown in Fig. 6 demonstrate that four neighbours have very different lag periods, and this is an indication that processes are occurring individual to each salt gland.

In comparing Figs. 3 and 4, it can be seen that the effect of an adverse potential gradient is to reduce the passive diffusion of ions, a process which takes place, parallel in time and space, to the active transfer. The gradient of 100 mV should theoretically reduce the diffusive flux by a factor of

$$\frac{zFE}{RT(1 - e^{zFE/RT})}$$

i.e., by a factor of 0.074 as calculated by the constant field equation[4]. This reduction of the passive flux by more than 90 % therefore unmasks the early stages of the active transfer, and demonstrates that there is also a clear lag period in the unidirectional transfer of Na^+ and Cl^-.

In view of the fact that the final level of transfer activity is determined by the external medium concentration and that the rise to a steady level is dependent upon a preliminary lag period incompatible in form with that obtained from a system of linear compartments, it seems reasonable to postulate that the transport mechanism is subject to metabolic induction. If this is the case, the action of Na^+ or Cl^-, or possibly both, is to control the synthesis of ion pumps; on the basis of current theories of gene action this could be achieved by control at the level of transcription of translation, assuming that the active ion-transport mechanism or its constituents are direct gene products. The tissue would therefore assemble sufficient ion-transporting machinery to cope with any particular salt load. There is good evidence that a similar process is operative in the avian salt gland for the controlled induction of (Na^+-K^+)-stimulated ATPase activity[5]. In plants which have been grown on seawater we can see that at the start of an experiment the transport mechanism seems to be operative but that it responds to 100 mM NaCl, implying that the cytoplasmic concentration is kept well below this level in the plant. Such a control process would certainly provide a mechanism for the euryhalinity of Limonium, which can tolerate the full spectrum of salinities from freshwater to full seawater and beyond.

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