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ION AND WATER TRANSPORT IN LIMONIUM

III. TIME CONSTANTS OF THE TRANSPORT SYSTEM

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SUMMARY

The analysis of $^{22}Na^+$ and $^{36}Cl^-$ efflux from Limonium leaf tissue has revealed several compartments for each ion. The transit of labeled ions from medium to glandular exudate is described by an exponential function with a single time constant for each ion. This transit half-time for an ion correlates with the half-time of the second tissue compartment, assumed here to be the cytoplasm. The transport pathway can thus be quantitatively described, and the gland cells must directly control the level of ions in this space.

INTRODUCTION

The salt gland of Limonium is a multicellular structure which transports Na^+ and Cl^-, amongst others, from some internal compartment in the leaf tissue to the exterior. The aim of this paper is to demonstrate that this compartment is the cytoplasm of the surrounding cells and to determine the half-time of filling of this space when subjected to a NaCl load. This analysis is required for two reasons. In the first place, step changes in the concentration of the medium usually involve changes in the pumping rates which attain steady values only after several hours; the analysis of these changes requires a knowledge of the time constants of the system before anything definite can be said about their nature. Secondly, it is of interest to know that the gland directly controls the ionic activity of the cytoplasm rather than that of the free space. Structurally the glands are connected to the cytoplasm of surrounding cells by numerous plasmodesmata which leave the gland complex at specific gaps in its insulating cuticle.

METHODS

All experiments were done with *Limonium vulgare*, cultured in the laboratory without salt water.

Efflux experiments

Limonium leaf discs, with lower cuticle removed by abrasion, were pretreated overnight in solutions of $^{22}NaCl$ or $Na^{36}Cl$ at 100 mM concentration; a disc was then

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quickly rinsed (1 sec) and put into a glass vial containing 100 mM NaCl solution maintained at 20° by a water jacket. Solution was drawn from this vial through a scintillation flow cell and returned to the bulk solution. The total solution volume was approx. 100 ml, and the conditions of turbulent flow caused excellent mixing. The counts/min in the washing solution were automatically recorded at 1-min intervals. At the end of the counting sequence the vial was removed; 10 g of solid NaCl were added, and a reflux condenser attached; the contents were than boiled for 30 min to extract all radioactive label. After refluxing the vial was returned to the flow system and the counts/min of the solution recorded; this was constant and represented the total initial radioactivity in the disc.

Effluxes into a salt-free medium were also studied, 200 mM mannitol solution being used as the washing solution to avoid any immediate osmotic effects.

Transit fluxes

Leaf discs were pretreated overnight in 100 mM NaCl solution and mounted as for a short-circuit experiment[1]; they were voltage clamped at zero and allowed to attain steady states. The solution in the inner chamber from which the glands transport ions, was then changed to 100 mM $^{22}NaCl$ or $Na^{36}Cl$ solution, and the outer chamber sampled for emergent radioactivity as a function of time. The rate of change of activity in the outer chamber represents the rate of change of specific activity in the leaf compartment to which the glands are directly connected. There are no concentration changes, merely the movement of isotopic label through the system in the steady state.

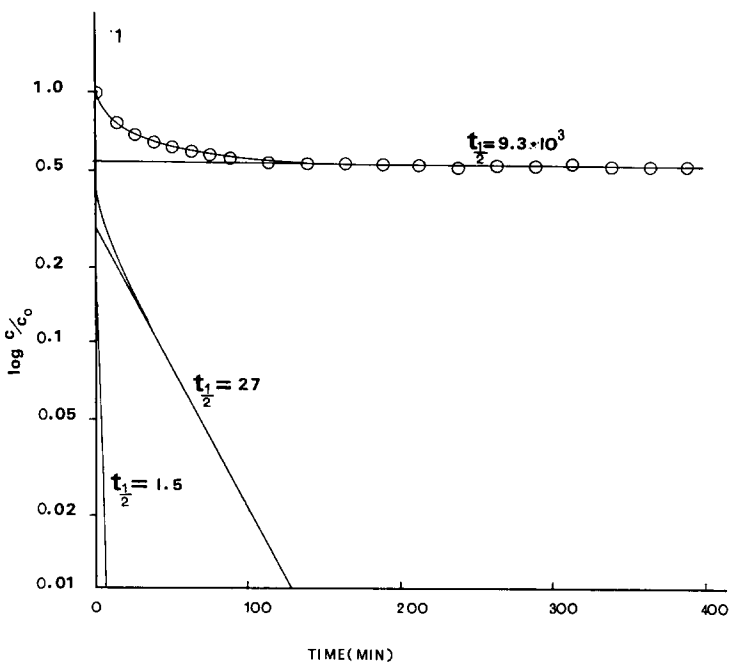

Fig. 1. The efflux of $^{22}Na^{+}$ from a sample disc, preloaded in 100 mM $^{22}NaCl$. The experimental curve has been broken down into three components by graphical subtraction. Ordinate is the logarithm of the remaining fraction.

RESULTS

In Fig. 1 is plotted the result of a Na^+ washout experiment in which the log fraction of the activity remaining in the disc is plotted against time; the Cl^- efflux has been similarly analysed but is not plotted here. The curve has been broken down graphically into several exponentials, and the total content can be represented by their sum[2];

$$c = \sum_{i} K_i e^{-k_i t}$$

TABLE I

ION SPACES IN LIMONIUM REVEALED BY EFFLUX ANALYSIS

Half times ($t_{1/2}$) for efflux into NaCl or water are shown, together with the total means. All times are in min.

Space	$^{22}Na^+$ *efflux*			$^{36}Cl^-$ *efflux*		
	$t_{1/2}(NaCl)$	$t_{1/2}(H_2O)$	$t_{1/2}(mean)$	$t_{1/2}(NaCl)$	$t_{1/2}(H_2O)$	$t_{1/2}(mean)$
1	2.0	1.22	1.5 ± 0.5	1.0	0.93	0.95 ± 0.2
2	27.3	33.1	29.2 ± 4.3	9.2	10.5	9.7 ± 2.1
3	3590	5990	4930 ± 1690	136.1	121.6	129.8 ± 26.3
4				4230	3150	3678 ± 1250

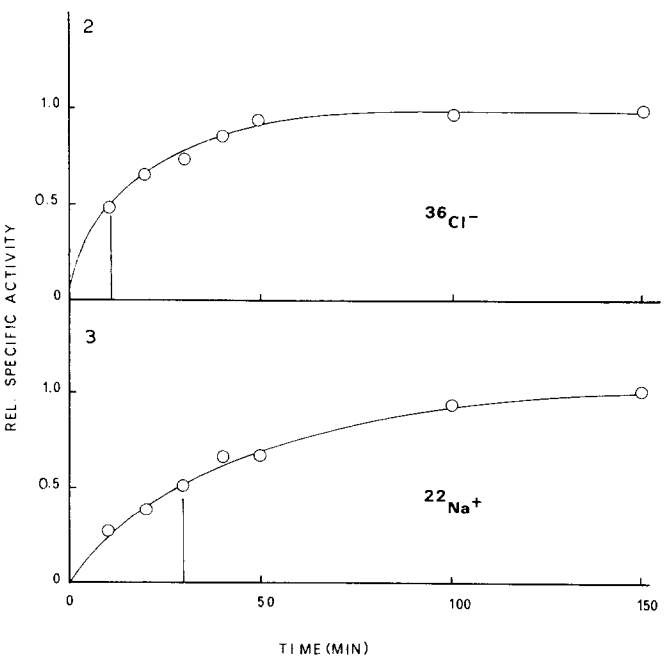

Fig. 2 (upper). Transit flux of $^{36}Cl^-$ across a steadily pumping disc short-circuited in 100 mM NaCl. The extruded fluid soon attains to the specific activity of Cl^- in the bathing medium (Table IV), so that transit rates are here represented as rises in specific activity. The half-time is indicated.

Fig. 3 (lower). Transit flux for $^{22}Na^+$ across a steadily pumping disc short-circuited in 100 mM NaCl. See legend of Fig. 2 for further details replacing Cl^- by Na^+.

each exponential component contributes to the total efflux from a loaded disc; the K's are the space contents and the k's are the respective rate constants. The half-times of the ionic compartments are given by the relationship $t_{½} = (\log_e 2)/k$. Some were obtained with 100 mM NaCl as the washing liquid and others with 200 mM mannitol solution (Table I).

In Figs. 2 and 3 are shown plots of the rate of transfer of $^{22}Na^+$ and $^{36}Cl^-$ from the inner chamber to the outer, *i.e.*, transit fluxes from $t = 0$. These figures represent integral plots of the curves obtained by sampling the outer chamber at fixed time intervals.

DISCUSSION

What are the leaf spaces of Table I? I would argue that they are the free space, the cytoplasm and the vacuole, *i.e.*, Spaces 1, 2 and 3 for Na^+ and 1,2 and 4 for Cl^-. This is in general agreement with other published data for plant cells, including giants where the cytoplasmic spaces can be individually sampled; the magnitudes of the half-times are quite similar[3–6]. The free space half-time has been calculated from the ionic diffusion coefficient as given by the relationship,

$$D = uRT$$

By taking

$$Dt/r^2 = 0.2$$

when the fractional distance from equilibrium,

$$c_t/c_0 = 0.5 \qquad \text{(ref. 7)}$$

the diffusion resistance factor (tortuosity) can be calculated for efflux from a sheet of thickness $2r$, equal to that of the leaf disc (0.017 cm) with one impermeable surface (Table II). The tortuosity factors for Na^+ and Cl^- are almost identical, indicating that the fastest space for the two ions is indeed the same compartment, the free space. The Cl^- space No. 3 remains unidentified; a candidate for this may well be the chloroplasts, for they constitute a membrane-bounded compartment in the cytoplasm occupying a substantial fraction of its volume.

From the analysis of transit fluxes for Na^+ and Cl^- (Figs. 2 and 3) it is apparent that the ion transfer mechanisms in the Limonium gland are sampling from a compartment in which the specific activity appears to rise as an exponential function:

$$S = S_{t=\infty}\,(1 - e^{-kt})$$

TABLE II

CALCULATED TORTUOSITY FOR Na^+ AND Cl^- IN THE FASTEST TISSUE SPACE

The tortuosity factor used here is the ratio of calculated to apparent diffusion half-times.

Ion	$t_{½}$		*Tortuosity factor*
	Calculated	*Apparent*	
Na^+	4.95	90	18.2
Cl^-	3.3	57	17.3

TABLE III

TRANSIT HALF-TIMES FOR Na^+ AND Cl^-

Ion	*Transit half-time (min)*
Na^+	26.1 ± 5.1
Cl^-	12.83 ± 4.2

By graphical inspection we can obtain the half-time of this rise, and this appears different for the two ions (Table III).

We can now see that these time constants show a correlation with those of the second slowest leaf compartment, *i.e.*, the cytoplasm, obtained from the efflux analysis; the active transfers of the two ions by the gland cells are therefore operating, as one would expect, from a common cytoplasmic space.

Following a step change in specific activity of the medium, the specific activity in this space will rise to a quasi-steady state given by the equation:

$$S = S_c (1 - e^{-k_2 t})$$

where S_c is the quasi-steady level. How near S_c approximates to S_0, the external activity at any time, depends upon the transfer constants of any slower spaces connected directly to Space 2, and upon their initial ion contents. In the excretory system of the mangrove *Aegialitis annulata* ATKINSON *et al.*[8] have adduced that there is probably a movement of Cl^- to the gland cells, *via* the free space of the leaf tissue, but their demonstration that the specific activity of the exudate is higher than that of the leaf tissue as a whole is of less importance than the demonstration that the specific activity of the fluid reaches a plateau at about 50 % of the activity of the medium. This is consistent with a transfer of ions from a cellular space in which S_c, the quasi-steady state specific activity, is held considerably below S_0 by equilibrium with the considerable Cl^- pool of the leaf. During open-circuit experiments the specific activity of Na^+ and Cl^- in the extruded fluid of Limonium is always close to S_0, Na^+ balancing Cl^-, Table IV. For the case where the second compartment is connected in the following manner to the vacuole

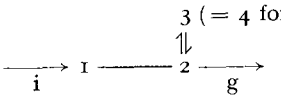

TABLE IV

SPECIFIC ACTIVITIES OF LIMONIUM GLANDULAR EXUDATE COLLECTED OVER 12 h, RELATIVE TO THAT OF MEDIUM (= 1.0)

Leaves were stood in beakers of radiosalt solution and the exudate washed off with water.

Ion	*Relative specific activity*
Na^+	1.01 ± 0.08
Cl^-	0.98 ± 0.15

i being the intake from the medium and g the glandular extrusion, the value of S_c is given in the steady state ($dS_c/dt \rightarrow 0$), by:

$$S_c = S_0 \frac{1}{\left(1 + \frac{J_3^*}{J_2^*} - \frac{J_3 S_0}{J_2^*}\right)}$$

where: J_3^* = radioisotope flux from Space 2 to 3, J_2^* = radioisotope flux from Space 2 *via* glands (g) and J_3 = net ion flux from Space 2 to 3. The specific activity of the free space (1) is assumed equal to S_0. J_2^* can be directly measured in the steady state, and J_3^* is given by the magnitude of the initial content of the vacuole during the washing out of a disc that has been preloaded for a known fraction of the vacuolar half-time. J_3^*/J_2^* has a mean value of near 0.1 for these experiments. S_c cannot therefore be lower than 0.9 S_0. The term $J_3 \cdot J_2^*$ is difficult to quantity, but its maximum value cannot exceed J_3^*/J_2^*; J_3 is almost certainly non-zero as the leaf discs are from low-salt plants and leaf tissue accumulates salt in spite of glandular activity, a fact seeming to require explanation. The experimental fact that the specific activity of the exudate approximates to S_0 therefore argues that the term $J_3 S_0/J_2^*$ is near maximum, and that the cytoplasmic ion concentration can be controlled by the glands independently of a net flux into the vacuole. The content of the latter is large enough to mask the contents of the other spaces. The fact that the short-circuit current also correlates well with the total net ion flux in the steady state, again indicates that the radioactive fluxes are true measures of the ion fluxes, and that the specific activity of the compartment from which the glands are pumping is similar to that of the solution in the inner chamber, *i.e.*, S_0.

When a Limonium disc of low-salt status is mounted in the chambers with 100 mM NaCl solution we may therefore assume that the salt concentration will rise in the cytoplasm with a time constant intermediate (*i.e.*, bounded) between those of Na^+ and Cl^-, with the following proviso: that the net influx rate of ions down a chemical concentration gradient into the cytoplasm, is equivalent to the influx rate of radioactive label in the steady state, *i.e.*, chemical fluxes and tracers fluxes are equivalent where kinetic parameters are concerned. From Table I it can be seen that replacement of the washing solution by 200 mM mannitol has no apparent effect on the half-times of the second spaces for either ion. This indicates therefore that the cytoplasm fills with salt as assumed, and that specific concentration effects (between 0 and 100 mM) on the net influx to this compartment are negligible.

To summarise we can say that both efflux analysis and the analysis of transit fluxes indicate that the transit half-times of both ions correlate with those of cytoplasmic filling, and that the half-times for salt loading and extrusion should therefore lie between these times. Knowledge of the kinetic constants of the process enables us to predict the form of the diffusive lag to be expected when the concentration of the medium is changed, an essential prerequisite to any analysis when metabolic effects are also present. It further indicates that the second tissue compartment is the cytoplasm, and that the gland cells directly control the ionic activity therein.

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