BBA 75041

ION AND WATER TRANSPORT IN LIMONIUM

I. ACTIVE TRANSPORT BY THE LEAF GLAND CELLS

A. E. HILL
Botany School, Cambridge (Great Britain)

(Received October 19th, 1966)

SUMMARY

Application of the Nernst equilibrium equation to the transport of ions in the leaf gland cells of *Limonium vulgare* indicates that Na^+, K^+ and Cl^- are actively transported out of these cells. Probing of the surface with constant-voltage pulses indicates that the gland cells are embedded in a relatively impermeable cuticle, whilst studies with alternating current show that the two surfaces behave differently. The results support the assumption that the gland-cell complex constitutes a mechanism for lowering ionic activities in the photosynthetic parenchyma. Various aspects of the process are briefly discussed.

INTRODUCTION

The genus Limonium in common with many other saltmarsh angiosperms and the tropical mangroves possesses glands on the leaves which exude concentrated salt solution; their function, like that of similar structures in many marine reptiles and birds, is partially to desalinate tissues which accumulate salts from sea water. The ion glands of *Limonium vulgare* provide an interesting example of a higher plant tissue which is directly amenable to electrophysiological studies. The work of SCHTSCHERBACK[1] and RUHLAND[2] on *Limonium gmelini* shows that the glands can extrude both electrolytes and non-electrolytes present in the basal medium, and in an interesting paper ARISZ *et al.*[3], have demonstrated that osmotic work is performed by the gland cells of *Limonium latifolium* whilst the process is sensitive to low temperature, respiratory inhibitors and light. No measurement of the electrochemical driving forces on the ions present has been made, however, and thus no specific ion-transport mechanisms have been described in these cells. The Limonium leaf thus offers a valuable approach to the study of ion and water transport in the photosynthetic parenchyma of a higher plant.

An analysis of the ionic concentrations in the glandular exudate under conditions where it is in equilibrium with its own vapour pressure is clearly desirable, and when this is combined with measurements of the transglandular potential the

driving forces on various ions can be defined. In this series of experiments the Nernst equation

$$\Delta E = -\frac{RT}{zF}\log_e\frac{c_0}{c_i}$$

has been used to interpret such results and although this equation applies only to systems whose passively distributed ions are in flux equilibrium, a movement against an electrochemical potential gradient from basal medium to exudate is a clear indication of active transport. The ion gland is embedded in a thick cuticle which can be shown to have a low ionic conductance not withstanding the numerous stomata; the glandular frequency is some 10–20% lower on the underside, and so both surfaces would be expected to have a similar conductance to ions, but this is not supported by experiment. When alternating current of various frequencies is passed through the leaf normal to its surface, the resistance is decoupled by a reactive element at quite low frequency, the impedance falling to 3% of its maximum value at 2000 cycles/sec. Removal of either cuticle by abrasion causes the impedance to fall, and removal of both cuticles creates a preparation whose impedance is independent of frequency over the same range. This indicates that the parenchyma of the leaf represents a zone of high conductivity which is presumably apoplasmic. An analysis of the impedance–frequency curves leads to a simple electrical analogue of the leaf in which the reactance is identified with that of the membranes of the gland cells (Fig. 2). On the basis of this analysis the leaf with lower cuticle removed represents a parenchymatic zone from which the ion-gland cells transport water and ions in a unidirectional manner. The analogy to an animal epithelial preparation is thus reasonably exact, and the pretreatment here involves little more than the dissection of such an epithelium from an animal.

METHODS

Limonium vulgare collected from an East Anglian saltmarsh was grown on soil, watered with tap water, in glass-topped chambers. The salt status of the plants was therefore very low, and leaf discs taken straight from the plant showed little secretion. Secretion was measured by running oil onto a leaf surface and observing the rate of change of diameter d, of the secretion drops with an eye-piece micrometer on a microscope stage. A plot of dd^3/dt against concentration of the pretreatment solution is shown in Fig. 1 for NaCl.

Potential difference studies

Pretreatment of leaf discs involved abrasion of the lower cuticle and immersion in aerated NaCl–KCl solution for 24 h. Discs were then rinsed in distilled water and blotted dry; small perspex rings with cover-glass lids 1 cm in diameter were cemented to the upper surface of the discs with vaseline and a disc of lens paper included in each chamber so formed. The discs were then laid in petri dishes on filter paper soaked in the pretreatment solution and left overnight in artifical light, a 60-W tungsten bulb at 50 cm. The lens tissues were then transferred to weighed, stoppered tubes and after weighing, the water was allowed to evaporate. The salts were eluted from each paper with 10 ml distilled water and Na^+ and K^+ assayed by flame photo-

metry. Cl^- was assumed to be the balancing anion, in the light of the demonstration by ARISZ *et al.*[3] that chlorides account for more than 98% of the osmotic potential of the exudate.

Potential differences between basal medium and secretion were determined between a reference electrode in contact with the filter paper and a 20 μ tip glass electrode filled with 3 M KCl in temporary contact with the exudate; these were connected by screened leads to a high-impedance millivoltmeter. The potentials recorded from this simple system were stable and reproducible.

Resistance studies

Discs were pretreated without removal of cuticle for 24 h in a medium representing sea water diluted 4–5 times, *i.e.* 100 mM NaCl; 10 mM $MgCl_2$; 2 mM KCl; 2 mM $Ca(NO_3)_2$. They were then clamped between two perspex chambers of 1.76 cm^2 cross section containing platinum black spirals at their end faces, and the impedance measured over the range 20 to 2000 cycles/sec (sine) with an a.c. bridge, using an oscilloscope to obtain balance by generating Lissajou figures. The disc was then removed and one of the cuticles abraded, after which the procedure was repeated. Finally the impedance was measured with both cuticles abraded, and then with no disc present at all. An equivalent circuit of the disc impedance is shown in Fig. 2, from which values of the reactance and resistance of the surfaces can be calculated. Measurement of the capacitance with an autobalance bridge at 50 cycles/sec gave a value in good agreement to that obtained from the frequency analysis.

The conductance of the leaf surface is the sum of the gland and cuticle conductances, and when considering passive ion fluxes it becomes of considerable interest to know the relative contributions of these two structures to the total conductance; consequently the resistance of the surface was mapped in detail. A leaf disc with lower surface removed was clamped onto a shallow perspex trough by a small cup, with a vaseline seal (Fig. 3). The underside of the disc was in contact with a basal solution of 100 mM KCl, whilst the cup was filled to a depth of 0.5 mm with a glycerol–brine solution, containing a wetting agent; this solution had a controlled conductivity and evaporated slowly. Under this solution a microelectrode of 1 μ tip diameter was used to inject square-voltage pulses of constant amplitude and 1 sec width at a frequency of 0.1 per sec. The basal solution was earthed *via* a salt bridge, calomel electrode and a 1 kΩ resistor, across which the current was monitored with a differential amplifier with 100:1 in-phase rejection. Under a long-range objective the surface was scanned with the electrode and the current pulses passing the ion glands, stomata and cuticle recorded.

RESULTS

The Nernst equation was used to calculate E_{Na}, E_K and E_{Cl} from the gradients of concentration over the gland. The potentials measured were small, ranging from 9 to 15 mV and in fact are little dependent on the ionic concentration external to the glands. The equilibrium potentials are displayed in Table I and these can be compared with the mean value of the potential, E_m.

The impedance values of the whole leaf disc are plotted in Fig. 2. The three curves represent the impedance of (a) the intact disc, (b) the disc with lower cuticle

TABLE I

COMPARISON OF TRANSGLANDULAR POTENTIALS WITH THOSE CALCULATED BY THE NERNST EQUATION

Ion	c_i/c_o *(mean)*	$\frac{RT}{zF} \log_e \frac{c_i}{c_o}$	*Potential difference (mV)*
Na^+	$\frac{33.3}{175.7}$	-38 ± 4	
K^+	$\frac{15.3}{85.7}$	-38 ± 6	-9 to -15
Cl^-	$\frac{48.6}{261.5}$	38 ± 4	

removed, and (c) the disc without cuticles. The resistance of the chamber and electrodes when filled with fluid was 300 Ω, and this is subtracted from the data. The complex impedance of the Limonium leaf is equivalent to a circuit shown in Fig. 2; having measured the low-frequency impedance of the disc with one or both cuticles removed it is possible to calculate the magnitude of the reactive element which shunts the resistance associated with the surfaces. An example will make this clear; the mean low-frequency impedance of the disc with lower surface removed is 11 kΩ. Removal of the remaining upper surface reduces the low-frequency impedance to

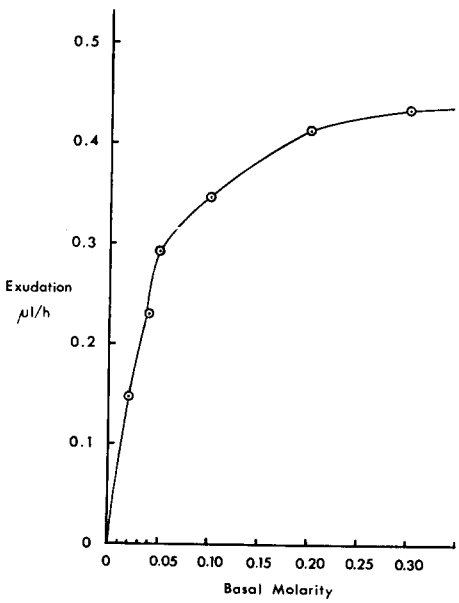

Fig. 1. Effect of pretreatment with increasing NaCl molarities on the volume exudation of Limonium.

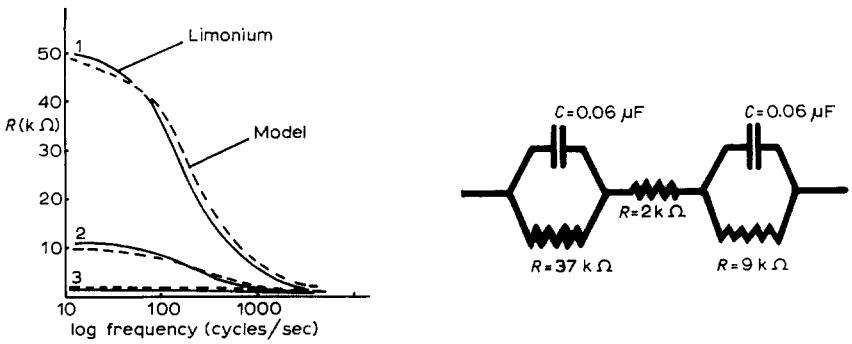

Fig. 2. Left: Solid line, impedance of disc plotted against frequency. 1. Whole disc. 2. Disc without lower cuticle. 3. Disc without cuticles. Right: Electrical analogue of the disc. Dotted line in left-hand figure represents frequency properties of the analogue.

2 kΩ, a value which is almost independent of frequency. The disc without lower surface is thus represented by the expression

$$Z = R_{\text{parenchyma}} + \frac{R_{\text{upper surface}}}{\sqrt{1 + \omega^2 C^2 R^2_{\text{upper surface}}}}$$

where

$\omega = 2\pi f$, f being the a.c. frequency; C = capacitance

Inserting the two R values this reduces to

$$Z = 2 + \frac{9}{\sqrt{1 + 100\,\omega^2 C^2}}$$

and thus C can be calculated from the data. If it is thus assumed that the reactance is wholly capacitative then at 2000 cycles/sec this is equivalent to 0.06 μF, or 0.034 μF/cm², the chamber cross section being 1.76 cm². The dependance of reactance upon frequency was not investigated but measurement of the capacitance with an autobalance bridge gave a value of 0.04 μF/cm² at 100 cycles/sec.

An analogue of the leaf disc (Fig. 2) was constructed from resistors and capicators, having the following values, representing those of the means: $R_{\text{upper surface}}$ = 9 kΩ; $R_{\text{parenchyma}}$ = 2 kΩ; $R_{\text{lower surface}}$ = 40 kΩ; capacitance (C) = 0.06 μF

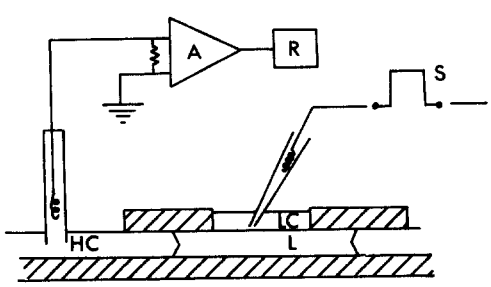

Fig. 3. Probing circuit. L, leaf disc; HC, high-conductance solution; LC, low-conductance solution; S, square-wave input; A, differential electrometer; R, recorder.

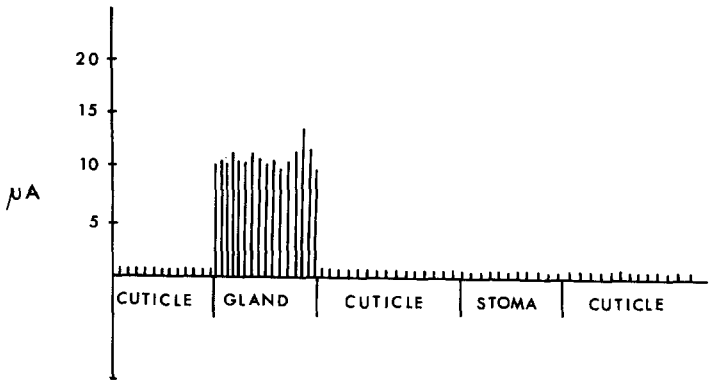

Fig. 4. Probing the surface with constant-voltage pulses. Ordinate, magnitude of the current pulse. Abscissa, surface topography (diagrammatic).

and its frequency characteristics were examined with the a.c. bridge circuit; the broken curves in Fig. 2 were obtained, showing that the fit is quite good. The current pulses passing various sections of the surface were obtained from the recorded traces of the amplifier output, of which a typical example is shown in Fig. 4, where the electrode has traversed a stoma and gland as indicated underneath. The current pulses passing the glands are 10 times the size of those passing cuticle or stomata, between which there is little difference. To calculate the conductance of the ion glands the ion conductance of the immersion liquid must be known, and this was determined with a conductivity bridge, which gave a value of 1.6 mhos/cm^2 at 21°. The glandular frequency on the sample of discs used was 573 per cm^2 and the mean resistance of the microelectrodes used, 330 kΩ. As a first approximation the contribution of the neighbouring glands and surrounding cuticle was ignored when calculating the conductance of a gland; when calculating the conductance of the cuticle however, the effect of the four nearest neighbours at a distance of $\sqrt{A}/\sqrt{2}$, where A is the mean surface area per gland, was taken into account. The correction required is very small, amounting to approx. 1%. The ion gland thus appears to be surrounded by cuticle whose conductivity is an order of magnitude smaller than the gland itself; as the cuticle has the greater area, however, amounting to roughly 100 times that of the gland, the greater part of the surface conductance must be due to the cuticle.

DISCUSSION

The calculated Nernst equilibrium potentials indicate that all three ions are actively transported out of the ion-gland cells, which seems to confirm that these do function as desalinators. There is no indication here whether these ion-transport mechanisms are electrogenic in nature, or to what extent the potential difference, which is small in this system, is controlled solely by the permeability coefficients as expressed by the Goldman equation. This can only be studied by short circuiting the disc and determining the total charge passing the ion cell membranes under conditions where the electrochemical driving force can be accurately controlled.

The a.c. impedance studies indicate that the upper and lower surfaces have different conductances and that this is not associated with differences in glandular

frequency, but must be due to a difference in the behaviour of the lower gland cells; at present no explanation can be given for this behaviour. Previous authors have commented on the extension of the cuticle around the gland-cell complex[3,4] and attributed it to the fact that the exudate leaves the gland under pressure and so mechanical containment of the cells is necessary. A more likely explanation is that the cuticle extension 'insulates' the complex by severely reducing the conductance pathway which would occur through the cell walls of the ion-gland cells.

It is perhaps profitable to outline some of the central problems which these interesting cells seem to pose, and to indicate further lines of research. It is of great interest to decide whether the ion pumps are neutral or electrogenic, and how the rate of pumping depends upon concentration of the major ions present at the transport site. Where is the transport site in the cell, and is it the same for both anions and cations? A study of the ionic compartments and half-times in the surrounding cells would provide valuable information here, possibly combined with an analysis of the driving forces on water and ions in them. Water may move over the ion cell membranes down a hydrostatic or osmotic gradient, or it may be transported electro-osmotically; are these driving forces adequate to account for the water fluxes?

The electron-microscopical evidence so far presented seems to confirm that the glands are connected to the neighbouring cells by numerous plasmodesmata, and that each cell contains numerous mini-vacuoles[5]; to what extent pinocytotic mechanisms of transfer must be investigated depends perhaps upon whether they can be seen in operation, and whether other postulated mechanisms of transfer seem to break down. The biochemical ramifications are considerable. The demonstration by ARISZ *et al.* that the transfer of chloride is light-dependent in these cells poses the problem as to whether the cation and anion transport are light-driven by separate mechanisms in higher plant cells as indicated for example by MACROBBIE[6], in a giant algal cell. Attempts to answer some of these questions will be presented in future papers.

ACKNOWLEDGEMENTS

I should like to express my thanks to Dr. D. C. SPANNER, Bedford College, London, for many helpful discussions. The work was supported by a Science Research Council Research Studentship and Research Followship.

REFERENCES

1 J. SCHTSCHERBACK, *Ber. Deut. Botan. Ges.*, 28 (1910) 30.
2 W. RUHLAND, *Jahrb. Wiss. Botan.*, 55 (1915) 409.
3 W. H. ARISZ, I. J. CAMPHUIS, H. HEIKENS AND A. J. VAN TORREN, *Acta Botan. Neerl.*, 4 (1955) 322.
4 R. J. HELDER, *Plant Physiol.*, 2 (1956) 468.
5 H. ZIEGLER AND U. LUTTGE, *Planta*, 70 (1966) 193.
6 E. A. C. MACROBBIE, *Biochim. Biophys. Acta*, 94 (1965) 64.