J. Membrane Biol. 170, 229–242 (1999)

The Journal of
Membrane
Biology

The Effect of an Extracellular Mucilage on the Response to Osmotic Shock in the Charophyte Alga *Lamprothamnium papulosum*

V.A. Shepherd, M.J. Beilby
School of Physics, Department of Biophysics, The University of New South Wales, NSW 2052, Australia

Received: 24 March 1998/Revised: 28 April 1999

Abstract. We have used current/voltage (*I/V*) analysis to investigate the role played by extracellular mucilage in the cellular response to osmotic shock in *Lamprothamnium papulosum.* Cells lacking extracellular mucilage originated in a brackish environment (1/3 seawater). These were compared, first with cells coated with thick (~50 μm) extracellular mucilage, collected from a marine lake, and second, with equivalent mucilaginous marine cells, treated with heparinase enzyme to disrupt the mucilage layer. Histochemical stains Toluidine Blue and Alcian Blue at low pH identified the major component of the extracellular mucilage as sulfated polysaccharides. Treating mucilage with heparinase removed the capacity for staining with cationic dyes at low pH, although the mucilage was not removed, and remained as a substantial unstirred layer. Cells lacking mucilage responded to hypotonic shock with depolarization (by ~95 mV), cessation of cyclosis, due to transient opening of Ca^{2+} channels, and opening of Ca^{2+}-activated Cl^- channels and K^+ channels. Cell conductance transiently increased tenfold, but after 60 min was restored to the conductance prior to hypotonic shock. Mucilaginous cells depolarized by a small amount (~18 mV), but Ca^{2+} channels failed to open in large enough numbers for cyclosis to cease. Likewise most Ca^{2+}-activated Cl^- channels failed to open and conductance increased only ~1.2-fold above the prehypotonic level. After 60 min conductance was less than the conductance prior to hypotonic shock. Heparinased mucilaginous cells recovered several aspects of the hypotonic response in cells lacking mucilage. These cells depolarized (by ~103 mV); cyclosis ceased, indicating that Ca^{2+} channels had opened, and conductance increased to ~4 times the value prior to hypotonic shock, indicating that Ca^{2+}-activated Cl^- channels opened. However, after 60 min, these cells had neither restored membrane potential (and remained at positive values), nor decreased their conductance. It was not possible to determine whether K^+ channels had opened. The heparinased cells recovered the normal hypotonic response of mucilaginous cells when heparinase was washed out. Apical seawater cells, which lacked mucilage, were unaffected by heparinase treatment. The results demonstrate that the presence of extracellular sulfated polysaccharide mucilage impacts upon the electrophysiology of the response to osmotic shock in *Lamprothamnium* cells. The role of such sulfated mucilages in marine algae and animal cells is compared and discussed.

Key words: *Lamprothamnium* — Hypotonic effect — Marine algae — Turgor regulation — Sulfated polysaccharide — Mucilage

Introduction

The salt-tolerant charophyte *Lamprothamnium papulosum* inhabits estuaries and coastal pools where the salinity can vary between extreme limits, from freshwater to several times the salinity of seawater. This is attributed to the ability of *Lamprothamnium* cells to regulate cell turgor pressure according to the osmolarity of the environment. Based on our initial study of the response to hypotonic shock (Beilby & Shepherd, 1996) we propose that three types of transporters are involved (Fig. 1*a* and *b*): (1) putative stretch-activated (SA) channels, (2) Ca^{2+}-activated Cl^- channels and (3) K^+ channels. Different current/voltage (*I/V*) and conductance/voltage (*G/V*) profiles are associated with each type of transporter (*see* Fig. 1*b* and *c*). The putative SA channels are of low conductance (or low density), voltage independent, and not inhibited with tetraethylammonium (TEA) or $LaCl_3$. The Ca^{2+}-activated Cl^- channels exhibit very large conductances with a maximum between −150 and 0 mV, accompanied by cessation of cytoplasmic streaming. The K^+ channels exhibit a "double bump" *I/V* profile, with resting potential difference (PD) near estimated E_K, and

conductance maximum between −100 and 0 mV. Negative conductance regions are often but not always observed, and result from strong dependence on the PD. It is difficult to distinguish between K^+ channels and Ca^{2+}-activated Cl^- channels on the basis of *I/V* curves alone, but we assume that the latter do not open in significant numbers if cytoplasmic streaming does not slow or cease.

Using pharmacological dissection (TEA as K^+ channel blocker, and $LaCl_3$ as Ca^{2+} channel blocker) we established that K^+ channels (3) and Ca^{2+}-activated Cl^- channels (2) open independently of each other. The Cl^- conductance diminishes within 30 min, and the K^+ conductance within 60–90 minutes (Beilby & Shepherd, 1996). However, when we measured the changing vacuolar ion concentrations over longer time courses, we found that the Cl^- and K^+ outflow continued for ~24 hr (Beilby, Cherry & Shepherd, 1999). A possible explanation is the sustained efflux of these ions through (1), SA channels which remain open for longer periods than transporters (2) and (3), which orchestrate the acute response and generate the large whole cell conductance (*see* Fig. 1). This hypothesis was supported by the finding of Bisson and Kirst (1980*a*), that turgor adjustments continued over several days following the acute response in *Lamprothamnium.*

The picture became more complex when we found that the "classic" hypotonic response, involving all three systems shown in Fig. 1, is restricted to young apical cells (Shepherd, Beilby & Heslop, 1999). The difference in the type of response to hypotonic shock was strongly correlated with the development of an extracellular layer of sulfated polysaccharide mucilage, which was thickest in older cells and cells from more saline enviornments (Shepherd et al., 1999). Hydraulic conductivity (Lp), was also significantly reduced in older, mucilaginous cells (Shepherd et al., 1999). The results showed that presence of the mucilage was correlated with diminution of the electrophysiological response to osmotic shock.

In this paper we investigate the contribution of the mucilage to electrophysiology of the hypotonic response by using the enzyme heparinase to manipulate the mucilage structure. We compare the hypotonic response in mucilage-free cells, cells coated in a significant (~20–50 μm) extracellular mucilage layer, and in cells coated in mucilage, but treated with heparinase. Our work is the first direct demonstration that an extracellular sulfated polysaccharide mucilage modifies the electrophysiology of the response to osmotic shock in a marine alga.

Materials and Methods

Plant Material

Lamprothamnium papulosum was collected from several sites in the Tuggerah Lakes system, Central Coast, NSW. Plants growing in water with a salinity equivalent to 1/3 seawater (1/3 SW) were collected from a lake adjacent to a golf course, and SW plants were collected from Chain Valley Bay on Lake Macquarie. The plants were all fertile. The cells of the SW plants were all 10 mm long or less. Only apical 1/3 SW cells were this small; the basal cells were cm in length and not used in experiments. The salinity was measured using a hand-held refractometer. Cells from 1/3 SW were maintained in aerated 1/3 Ocean Nature (ON) medium (Beilby & Shepherd, 1996) and within a week produced new apices, which were harvested for experiments. SW plants had poor survival rates in ON and were kept in an aerated 1:3 mixture of lakewater/ON at pH 7.5. The K^+, Na^+ and Cl^- concentrations of seawater/ON were 16 mol m^{-3}, 350 mol m^{-3} and 400 mol m^{-3}, respectively; Ca^{2+} concentration was taken to be close to that of ON, 10.4 mol m^{-3}. Prior to experiments cells were cut from the plants and allowed to recover overnight.

Heparinase Treatment

Heparinase III (activity 200–600 units/mg; bovine heparan sulfate as substrate) was purchased from Sigma Chemical, St. Louis, MO. The powder was dissolved in millipore-filtered reverse osmosis water. Cells were placed into adjacent sterile tissue-culture chamber wells in equal volumes of millipore-filtered SW. Droplets of the heparinase solution were added by microsyringe to the SW to a final concentration of 2.4×10^{-3} International Units heparinase/cm^3 of SW. The pH was 7.5, and the temperature was 25°C, which are the optimal conditions for the enzyme; 18 hr was found to be the optimal treatment time.

In some experiments we tested the effect of washing out the enzyme overnight. The survival rate of the heparinased cells overnight following hypotonic and voltage-clamping experiments was similar to that of untreated cells, ~20%. Survivors were subjected to further hypotonic challenge, from 3/4 to 1/2 SW, an equivalent drop in osmolarity of 268 mosmol kg^{-1}.

The majority of apical SW cells, with only thin mucilage (Shepherd et al., 1999) were too small to fit into our apparatus, and there were insufficient cells to make a statistical comparison with the mucilaginous SW cells. Out of interest, we treated the largest apical SW cells with heparinase and subjected them to hypotonic treatment as described for SW cells.

Electrophysiology

Only healthy cells with vigorous streaming rates (30–100 μm/sec) were used. Cells were mounted in a 3-compartment chamber as previously described (*see* Fig. 1; Beilby, Mimura & Shimmen, 1997). Silicon grease was used to seal the cells into the chamber and to insulate the chambers from one another. Seal integrity was verified after experiments by checking whether neutral red diffused between compartments. The heparinased cells were mounted, as with the untreated controls, in SW. The chamber was substage illuminated with an obliquely oriented fiber-optic light source, giving a dark-field effect. Cells were observed using a dissecting microscope with maximum magnification 120×. Cells were compartment-clamped since they were too fragile to be space-clamped (Beilby & Shepherd, 1996). We did not correct for the nonuniform nature of the clamp, since the errors associated with compartment clamping are significantly less than the statistical scatter of the data (Beilby et al., 1997).

Microelectrodes were filled with 0.5 kmol m^{-3} KCl. One microelectrode served as the reference, the other for recording the cell membrane potential difference (PD). The recording microelectrode was inserted into the center of the cell when this was possible. Steady PDs

were obtained after 20–30 min when the medium was refreshed by hand at regular intervals.

The *I/V* and *G/V* characteristics were obtained by voltage clamping the PD across the cell membrane using a bipolar staircase, as previously described (Beilby, 1990; Beilby & Shepherd, 1996). The pulses were each 60 msec wide and membrane PD and current points were logged each millisecond. Conductance/voltage (*G/V*) characteristics were obtained as previously described (Beilby, 1990; Beilby & Shepherd, 1996), by fitting polynomials to the current/voltage (*I/V*) data and differentiating. We applied the clamp voltages cautiously to avoid damaging the cells. Beginning with a window of −50 to −250 mV, we stepped up to a maximum window of 50 to −350 mV. A chart-recorder (Riken-Denshii, Tokyo, Japan) was used to record the membrane PD throughout the experiments. These records were used to measure rates of depolarization.

The Hypotonic Response

The hypotonic response was provoked by a change in osmolarity of the bathing medium. In 1/3 SW cells, the osmolarity was halved to 1/6 SW (a drop of 179 mosmol kg^{-1}); this was the method employed in past studies (Beilby & Shepherd, 1996). SW cells were subjected to a drop in osmolarity from SW to 3/4 SW (a drop of 268 mosmol kg^{-1}). We expected a minimal response to this (*see* Shepherd et al., 1999). However it was an appropriate concentration change to reveal any difference between the response of the heparinased and SW cells.

The time course of turgor regulation and associated ion effluxes is well known (Bisson & Kirst, 1980*b*) and we previously dissected the time course of the rapid events in the first hour (Beilby & Shepherd, 1996). Experiments were designed to include the critical time intervals. We voltage-clamped the cells in steady state and then in intervals during the first 10, 30, 40 and 60 min following hypotonic shock. We use the terminology G_s for the conductance at the resting PD, prehypotonic shock, G_{rev}(initial) for the conductance at the reversal PD, ~10 min posthypotonic shock, G_{rev}(30) for the conductance at the reversal PD after ~30 min and G_{rev}(60) for the conductance at the reversal PD after ~60 min. The *I/V* and *G/V* characteristics and cell PD were also measured for 1–3 hr after hypotonic treatment. We did not attempt to voltage clamp during rapid changes in membrane PD.

Histochemical Staining of Extracellular Mucilage

The histochemical stains Toluidine Blue at pH 1 and Alcian Blue at pH 1 were used to detect sulfated polysaccharides in the extracellular mucilage. Only the sulfate groups are ionized and stain at low pH (0.5–1; McCully, 1970) and neutral (carboxylated) polysaccharides do not stain. Toluidine Blue (0.05%) was made up in 0.05 M KCl/HCl buffer, pH 1, for detection of sulfated polysaccharides (O'Brien & McCully, 1981). Alcian Blue (0.05%) was made up in KCl/HCl buffer at pH 1. Cells were stained for 2–5 min, rinsed and mounted in original medium (without heparinase) for observation using a Leitz Orthoplan microscope. Photographs were taken on Kodak EPY64T professional film rated at 64 ISO. The staining reactions of mucilaginous SW cells, heparinased mucilaginous SW cells, some 1/3 SW cells, and apical SW cells were compared. Staining reactions were scored from +++++ (70–90% of the layer stained) to ++++ (50–60% of layer stained) to +++ (30–40% of layer stained) to ++ (10–20% of layer stained) to very weak staining (less than 10% of layer stained) or − (no staining). The scores were determined from photographic records and visual measurements using an eyepiece micrometer.

In some experiments we tested whether the staining reactions, lost due to heparinase treatment, were restored if heparinase was subsequently removed. Since the staining kills the cells, we could not use the same cells throughout. Mucilaginous SW cells were heparinased as described, then removed overnight from the heparinase solution, and then stained with Toluidine Blue at pH 1.

The thickness of the extracellular mucilage was measured on unstained cells using dark-field optics, which gave excellent resolution. Sharp focus on the edge of the mucilage of a cylindrical cell equates to the central plane and the actual thickness of the mucilage. Measurements were made using an eyepiece micrometer and from 15 × 10 cm photographic prints. The mucilage was not completely even and 5 measurements were made for each cell and averaged. Cells where the mucilage was much thicker on one side or very patchy were discarded.

Results

I/V and G/V Curves in the Steady State

1/3 SW cells harvested from the tips of plants lacked extracellular mucilage or had very thin mucilage (Table 1). In steady state the cells exhibited the usual range of *I/V* and *G/V* profiles, ranging from hyperpolarized pump state (Fig. 2*a* and *b;* ▲), to linear "leak" state, and "double bump" K^+ state (*see* Figs. 1 and 3 of Beilby and Shepherd, 1996). The mean resting PD was ~−138 mV (Table 2). The mean resting conductance, G_s, was 2.2 Sm^{-2} (Table 2), similar to that found previously (Beilby & Shepherd, 1996).

SW cells had thick extracellular mucilage containing sulfated polysaccharides (Table 1). *I/V* and *G/V* profiles were characteristic of the K^+ state or had slightly nonlinear *I/V* and *G/V* profiles (Fig. 3*a* and *b;* ▲); with mean resting PD ~−90 mV (Table 2). The mean G_s (17 Sm^{-2}) was significantly greater than that of 1/3 SW cells (Table 2). We have previously found hyperpolarized SW cells in pump state with similarly high conductances (Shep-

Table 1. Staining reactions for sulfated polysaccharides in *Lamprothamnium* cells

Cell type	Mucilage thickness (μm)	Intensity of staining
1/3 SW	0.8 ± 0.2 (n = 5)	−
Apical SW	6.6 ± 0.8 (n = 4)	+
Basal SW	44 ± 3.2 (n = 10)	+++++
Hep-SW	36 ± 3.3 (n = 10)	+
Hep-SW-washout	34.2 ± 4.3 (n = 10)	++++

Staining reactions with Toluidine Blue at pH 1 of marine cells (SW), SW cells treated with heparinase enzyme (hep-SW), hep-SW cells following washout of heparinase, several apical SW cells, and 1/3 SW cells are shown. Mean and standard error of the thickness of extracellular mucilage in these cells are also shown. Staining intensity is rated as +++++ (~70–90% of layer stained), with ++++ corresponding to ~50–60% of layer stained and +++ to 30–40%. Weak staining (10–20% of layer stained) is represented as ++, and barely detectable staining as +. Absence of staining is represented with −.

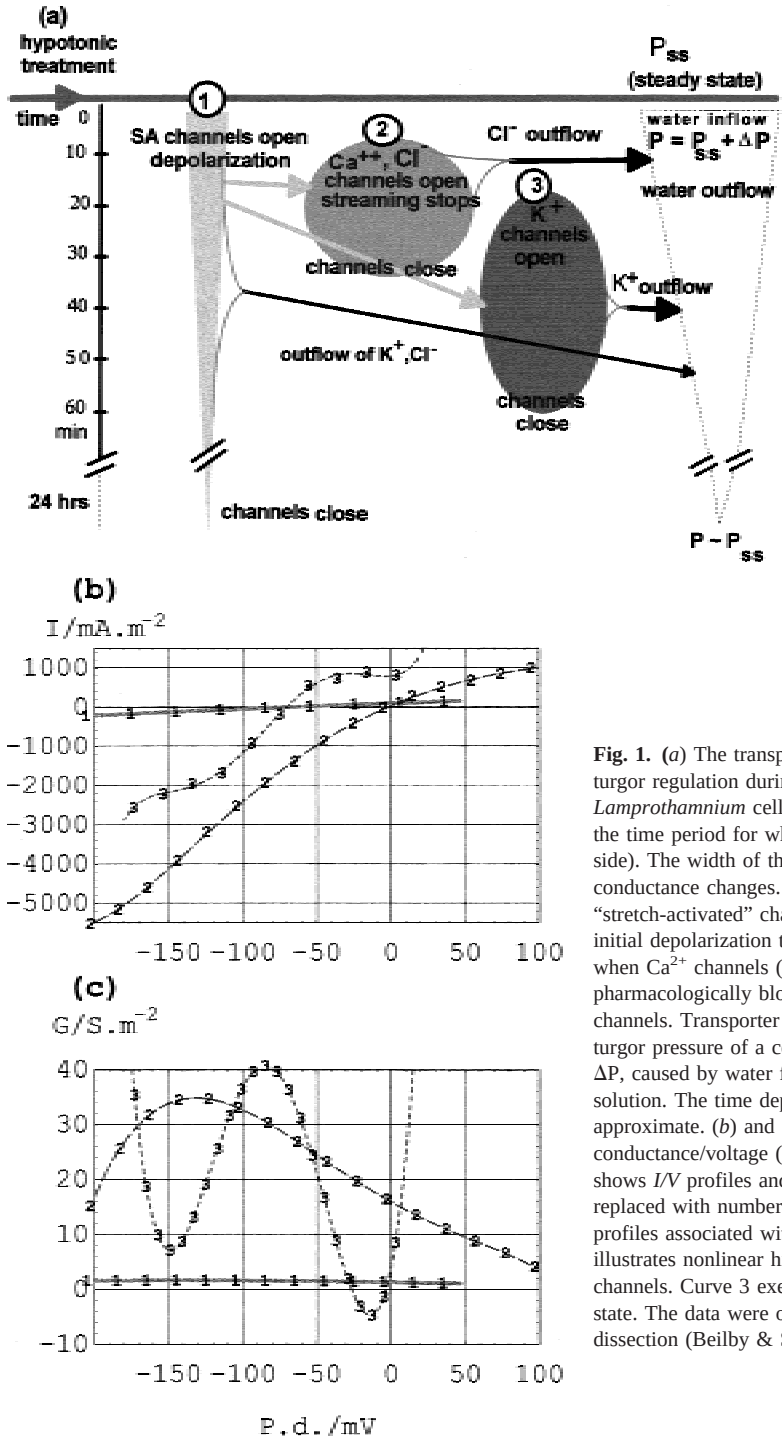

Fig. 1. (*a*) The transporters (1, 2 and 3) we hypothesize to be involved in turgor regulation during the response to hypotonic challenge in *Lamprothamnium* cells. The height of the shaded areas is in proportion to the time period for which they are activated (*see* time-scale on left-hand side). The width of the shaded areas is proportional to the magnitude of the conductance changes. Transporter 1 comprises the putative "stretch-activated" channels, which we hypothesize are responsible for the initial depolarization that occurs in response to hypotonic challenge, even when Ca^{2+} channels (and Ca^{2+}-activated Cl^- channels) and K^+ channels are pharmacologically blocked. Transporter 2 represents the Ca^{2+}-activated Cl^- channels. Transporter 3 represents the K^+ channels. P_{SS} is the steady state turgor pressure of a cell, which increases to P by the additional pressure, ΔP, caused by water flowing into the cell when it is exposed to hypotonic solution. The time dependencies of the conductances and water flow are approximate. (*b*) and (*c*) Different current/voltage (*I/V*) and conductance/voltage (*G/V*) profiles associated with the three transporters. (*b*) shows *I/V* profiles and (*c*) shows *G/V* profiles. The data points have been replaced with numbers in accordance with 1(*a*). Curve 1 indicates the flat profiles associated with putative stretch-activated channels. Curve 2 illustrates nonlinear high conductance profiles of Ca^{2+}-activated Cl^- channels. Curve 3 exemplifies the "double bump" profiles of the typical K^+ state. The data were obtained from 1/3 SW cells by pharmacological dissection (Beilby & Shepherd, 1996).

herd et al., 1999) and the apparent large difference in mean PD of 1/3 SW and SW cells was not typical of all batches of cells.

Heparinased SW cells retained a thick mucilage coating, which had lost its capacity for staining (Table 1). The cells gave *I/V* profiles of either "leak" (Fig. 4*a* and *b;* ▲) or K^+ state, similar to the untreated SW cells. The mean G_s of heparinased SW cells was 3.4 times the G_s of untreated SW cells while the mean PD was not significantly different (Table 2).

Response to Hypotonic Challenge of Heparinased and Untreated Cells

One-third SW cells had negligible extracellular mucilage layers (Table 1). These cells gave the typical re-

Table 2. Changes in cell conductance during hypotonic treatment of *Lamprothamnium* cells

Cell treatment	Resting PD (mV)	G_s (Sm^{-2})	G_{rev} (initial) (Sm^{-2})	$G_{rev}(30)$ (Sm^{-2})	$G_{rev}(60)$ (Sm^{-2})
1/3 SW; lacking mucilage	−138 ± 1.3 (n = 5)	2.2 ± 1 (n = 5)	24.6 ± 2 (n = 4)	8.6 ± 4.4 (n = 4)	1.8 ± 0.4 (n = 4)
SW with mucilage	−93.8 ± 0.6 (n = 9)	17.2 ± 2.1 (n = 9)	20.9 ± 4.7 (n = 6)	13.1 ± 3.1 (n = 4)	12.4 ± 3.1 (n = 4)
Heparinased SW	−92.7 ± 1 (n = 6)	55.2 ± 1 (n = 6)	216.7 ± 3.5 (n = 5)	209.5 ± 4.7 (n = 2)*	168.6 ± 3.2 (n = 4)*

* These figures are underestimates because in some cells the magnitude of current flowing across the membrane was so large that its measurement was beyond the scope of the apparatus: i.e., G was greater than ~450 Sm^{-2}).

The conductances of 1/3 SW cells (lacking extracellular mucilage), mucilaginous SW cells, and heparinase-treated SW cells during the time course of hypotonic challenge are shown. The SW and heparinased SW cells were from the same thickly mucilaginous population. The resting PD and G_s are the mean membrane PDs and mean conductances, including standard errors, in either 1/3 SW of SW. G_{rev}(initial) is the conductance measured when the PD had stabilized (3–9 min) following hypotonic treatment, with 1/6 SW (1/3 cells) or 3/4 SW (SW cells). $G_{rev}(30)$ and $G_{rev}(60)$ are the mean conductances between 30–40 and 60–70 min in hypotonic solution.

sponse (Beilby & Shepherd, 1996) to hypotonic challenge: depolarization by ~100 mV; an average tenfold increase in conductance; and cessation of cytoplasmic streaming, followed by gradual recovery (Table 1, Fig. 2*a* and *b;* ●, △ and x). All three processes shown in Fig. 1 were involved, with activity of Ca^{2+}-activated Cl^- channels peaking in the first 10 min (Fig. 2*a* and *b;* inset, ●) and declining as the K^+ channel activity peaked, at 30–40 min (Fig. 2*a* and *b;* △). After 60 min the cells remained depolarized with *I/V* profiles close to linear, that is, "leak state" (Fig. 2*a* and *b;* x).

Table 2 shows the time course of average conductance changes in 1/3 SW cells. The average changes in conductance (G_{rev}) were first; G_{rev}(initial) increased 11-fold over G_s; followed by a gradual decline with $G_{rev}(30)$ being 3.9 times G_s and $G_{rev}(60)$ declining to 0.8 times G_s. This decline was consistent with the time course of turgor regulation (Bisson & Kirst, 1980*b*) and the expected decrease in *G* in more dilute media (Shepherd et al., 1999).

The onset of the depolarization was very rapid, between 30 and 40 sec for all 1/3 SW cells. The initial rate of depolarization (Table 3) was also very rapid (~+26.7 mV/min). Streaming ceased within 60 sec, coinciding with the initial rapid depolarization (Table 3). Cells reached a stable PD after 5 min. This coincided with the recovery of cytoplasmic streaming. Thereafter, the 1/3 SW cells slowly hyperpolarized (Table 3) as $G_{rev}(30)$ and $G_{rev}(60)$ declined. The cells developed a PD close to estimates of E_K (Table 3, Fig. 2*a* and *b;* △ and x).

SW cells had thick extracellular mucilage layers (44 ± 3.2 μm; Table 1). Fig. 3 (*a* and *b*) shows *I/V* and *G/V* curves of the hypotonic response in a mucilaginous SW cell. When transferred to 3/4 SW the cell depolarized (Fig. 3*a* and *b;* ●); cytoplasmic streaming did not slow, and initial G_{rev} was twice G_s. The *I/V* and *G/V* profiles exhibited the "K^+ state" (Fig. 3*a* and *b;* ●, △ and x). The *I/V* and *G/V* profiles remained characteristic of the K^+ state, as $G_{rev}(30)$ and $G_{rev}(60)$ progressively declined. This behavior was typical of the SW cells.

Table 2 shows the time course of changes in average G_{rev} in SW cells. In contrast to 1/3 SW cells G_{rev}(initial) increased only 1.2-fold over G_s. $G_{rev}(30)$ declined to 0.8 times G_s and $G_{rev}(60)$ declined to 0.7 G_s (Table 2).

For SW cells, the cell PD did not significantly change in the first minute following introduction of hypotonic solution and cytoplasmic streaming did not slow or cease (Table 3). Most (86%) SW cells transiently hyperpolarized (range, 7 to 36 mV) one minute after transfer to hypotonic solution (Table 3). The hyperpolarization lasted ~1 min including an equal and opposite decay to the resting PD, which was immediately followed by steady depolarization (Table 3) of, on average, only 17 mV, at a relatively slow rate (~+4.8 mV/min). Streaming was unaffected throughout. The PD remained close to estimated E_K (Table 3).

Heparinased SW cells had similarly thick mucilage layers (36 ± 3.3 μm; Table 1) prior to treatment. Overnight treatment did not visibly change mucilage thickness. The hypotonic response of heparinased SW cells was very different from that of SW cells (Fig. 4*a* and *b*). G_s was somewhat less (Fig. 4*b;* ▲) than the average for heparinased cells (Table 2). Three–five min after transfer to 3/4 SW, the heparinased cell depolarized to a positive value (+21 mV), in contrast to both 1/3 SW and SW cells, and stopped cytoplasmic streaming (Table 3). This behavior was typical of heparinased SW cells (Tables 2 and 3). After 14.5 min, the cell remained very depolarized (Fig. 4*a* and *b;* ●), as was typical of heparinased SW cells (Table 3). The cell had very large G_{rev}(initial) although cytoplasmic streaming recovered. This depolarized and highly conductive state continued for over an

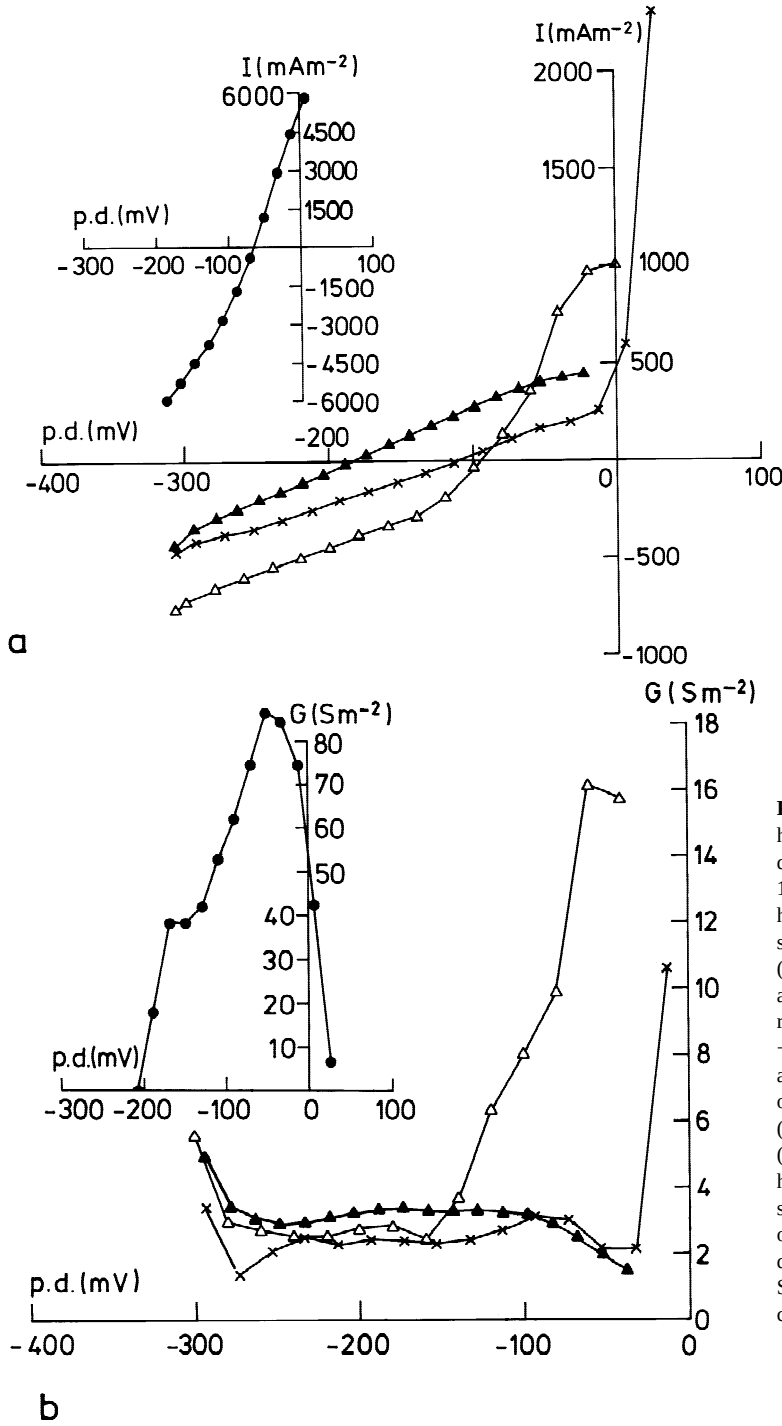

Fig. 2. *I/V* (*a*) and *G/V* (*b*) curves showing the hypotonic response of an apical 1/3 SW cell with no detectable extracellular mucilage, going from 1/3 SW to 1/6 SW. (▲). In the resting state, the cell was hyperpolarized (PD-182.53 mV). The cell was in pump state, shown by the sigmoidal curve. G_s was 3.4 Sm^{-2}. (●) (Inset). The cell began to depolarize immediately after transfer to 1/6 SW. Streaming ceased at 2 min and recommenced at 3 min. At 5 min the PD stabilised at −66.86 mV, and G_{rev} (initial) was 70 Sm^{-2}. The *I/V* and *G/V* profiles show predominantly Cl^- channel opening, with a contribution from K^+ channels. (△). The cell conductance had declined after 26 min (G_{rev}(30) was 8 Sm^{-2}) with the PD beginning to hyperpolarize (PD was −95.36 mV; close to E_K under similar conditions) with K^+ state ("double bump") form of the *IV* and *GV* profiles. (x). The cell conductance declined to less than G_s after 52 min (G_{rev}(60) was 2.9 Sm^{-2}), with the PD stable at −108.46 mV, and the *I/V* curve flattening out, with transporter (1) still active.

hour with G_{rev}(30) (Fig. 4*a* and *b;* △) and G_{rev}(60) (Fig. 4*a* and *b;* x) remaining large. This was typical of heparinased SW cells (Tables 2 and 3). The K^+ state was not detectable because it was swamped by the positive PD and large conductance. However the "shoulder" in the *I/V* and *G/V* profile in Fig. 4*a* and *b;* x) suggests that K^+ channels did open.

The average G_{rev}(initial) in heparinased SW cells increased on average ~3.9-fold over G_s (Table 2); this is an underestimate because *G* was too large to measure in some cells; that is, $G > \sim 450$ Sm^{-2}. In contrast to the 1/3 SW and SW cells, conductance was not recovered. G_{rev}(30) was still ~4 times > G_s and G_{rev}(60) was still 2.5 times > G_s. The cells retained the positive PDs

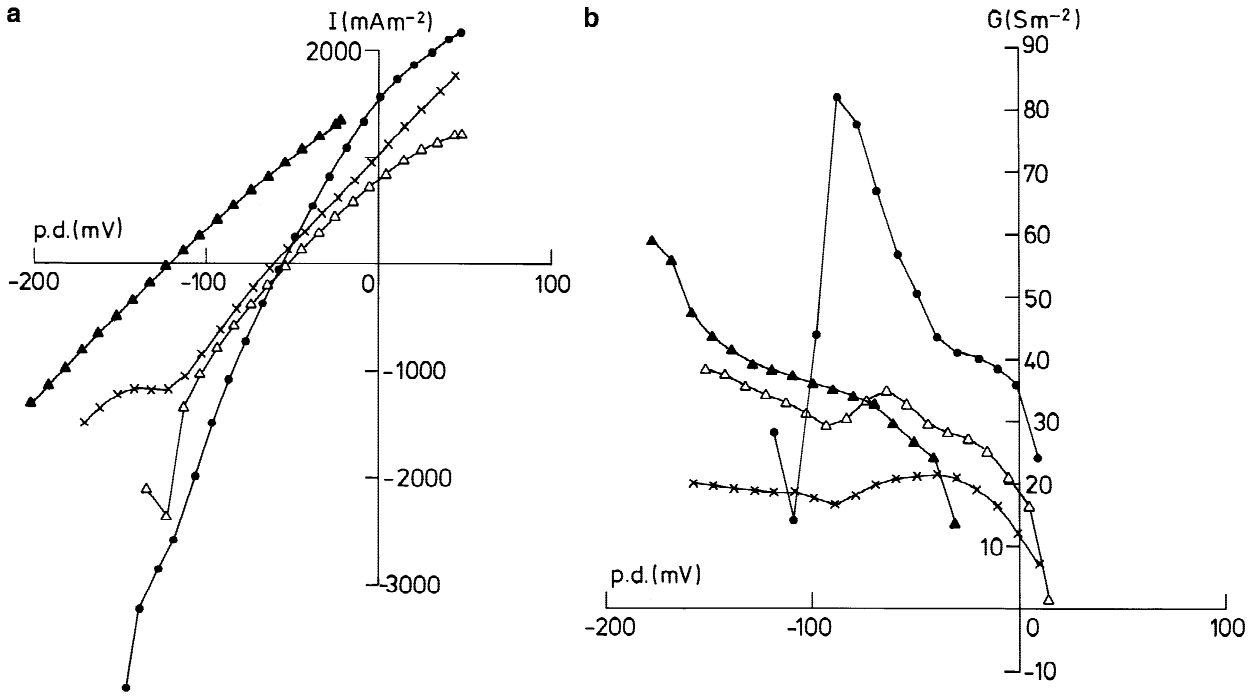

Fig. 3. *I/V* (*a*) and *G/V* (*b*) curves showing the hypotonic response of a basal SW cell with thick extracellular mucilage (30 μm), going from SW to 3/4 SW. (▲). In SW the cell was mildly hyperpolarized with resting potential −121.2 mV (more negative than E_k) and G_s of 15 Sm^{-2}. The *I/V* profile is not strictly linear (*see G/V* curve) and has an electrogenic component. (●). After 6 min in 3/4 SW the cell had depolarized to −55.2 mV and G_{rev}(initial) doubled to 32 Sm^{-2}. Cytoplasmic streaming did not cease or slow. The *I/V* and *G/V* profiles are predominantly K^+ state. (△). After 34 min/ in 3/4 SW, the PD was similar, −52.6 mV, but G_{rev}(30) had declined to 19 Sm^{-2}. The *I/V* and *G/V* profiles indicate the cell remained in the K^+ state. (x). After 62 min in 3/4 SW, the cell had hyperpolarized to −61.3 mV and G_{rev}(60) was 17 Sm^{-2}, close to G_s. The cell remained in the K^+ state.

and large conductances for over an hour. These PDs were close to estimates of E_{Cl} (Table 3).

In the first 3 min, the heparinased cells showed a blend of the responses of 1/3 SW and SW cells (Table 3). Rapid depolarization in the first minute (by ~+28 mV/ min) was not accompanied by streaming cessation. Between 1–2 min, the cells transiently hyperpolarized, as did SW cells. This was followed, between 2–3 min, by very rapid depolarization (~+41.3 mV/min). The cells depolarized by up to 160 mV, going to zero or even to positive values (Table 3). Streaming cessation occurred at this point. It was delayed by 2 min compared to 1/3 SW cells, and was prolonged (lasting between 5–7 min; Table 3).

Recovery Following Washout of Heparinase

SW cells given a further osmotic step from 3/4 SW to 1/2 SW 12 hr later had a minimal response, similar to that shown in Fig. 3 (*data not shown*). A similar response occurred in the heparinased cells when heparinase was removed for 12–18 hr. Figure 5 shows *I/V* and *G/V* curves from the same heparinased cell as Fig. 4, going from 3/4 SW to 1/2 SW in the absence of the enzyme. In its resting state in 3/4 SW the cell had a PD close to E_K, ~−80 mV (Fig. 5*a* and *b;* ▲) which was very different to the PD of +10 mV it had 60 min after hypotonic shock following heparinase treatment. The cell streamed vigorously. G_s was 37.5 Sm^{-2}, far less than the conductance after hypotonic shock following heparinase treatment (295 Sm^{-2}). When transferred to 1/2 SW the cell continued cytoplasmic streaming, depolarized slowly, and entered the K^+ state (Fig. 5*a* and *b;* ●). Thus, the cell recovered a response within the normal range of SW cells. G_{rev}(60) was 33 Sm^{-2}, approaching G_s, (Fig. 5*a* and *b;* △). After 99 min (Fig. 5*a* and *b;* x) the PD had hyperpolarized further to −60.9 mV, and G_{rev} had declined further, to 21 Sm^{-2}, less than G_s. Thus the cell recovered a hypotonic response typical of SW cells if heparinase was removed.

Hypotonic Response of Heparinased Apical SW Cells which were Deficient in Mucilage

Figure 6 shows the hypotonic response of an apical heparinased cell with mucilage layer only 2.4 μm thick. The

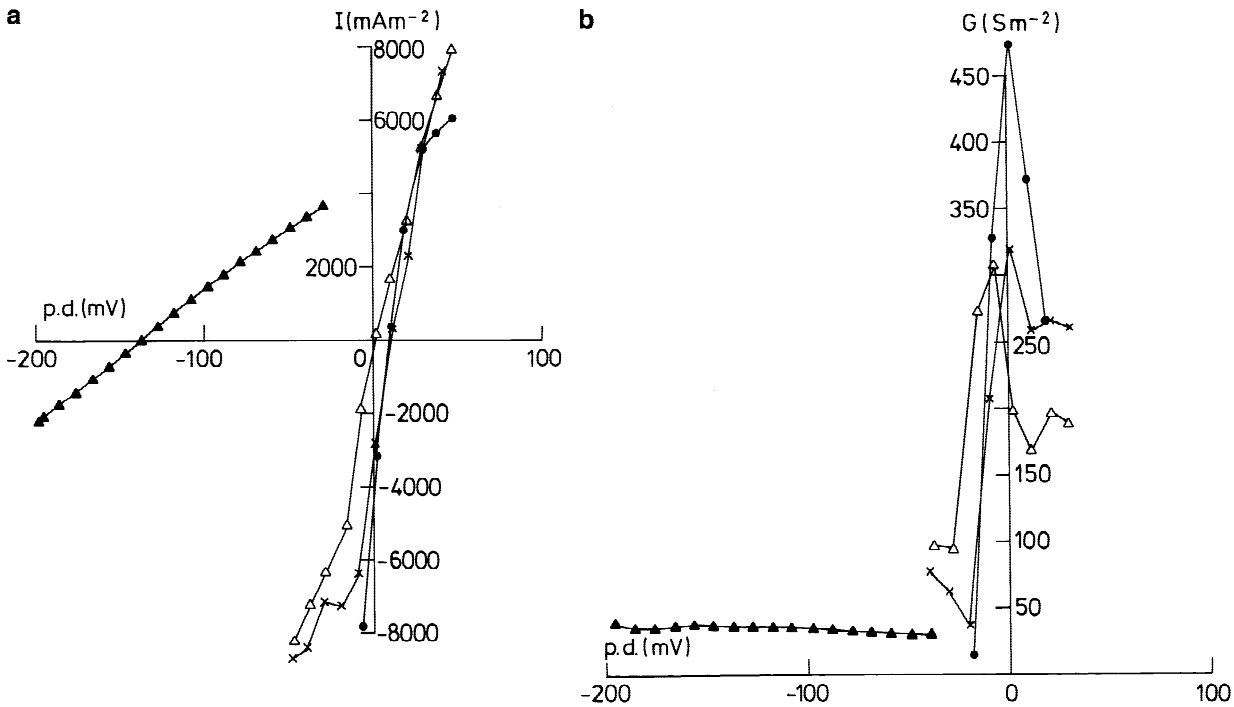

Fig. 4. *I/V* (*a*) and *G/V* (*b*) curves showing the hypotonic response of a basal SW cell with thick extracellular mucilage (42 μm), treated overnight with heparinase. (▲). In SW the treated cell was mildly hyperpolarized, with PD −137.9 mV, and G_s of 37.5 Sm^{-2}, larger than in the control cell in Fig. 3. The linear *I/V* profile indicates the leak state but the hyperpolarized PD suggests there may be a contribution from the pump. (●). On introduction of 3/4 SW the cell immediately and rapidly depolarized. After 14.5 min in 3/4 SW, cytoplasmic streaming had stopped for 5 min, and recovered, and the cell had a positive PD of 9 mV. G_{rev}(initial) had increased to 450 Sm^{-2}. (△). After 46 min in 3/4 SW the cell remained depolarized, with zero PD, although cytoplasmic streaming occurred at the normal rate. G_{rev}(30) at this PD remained very high, 225 Sm^{-2}. (x). After 60 min in 3/4 SW the cell was further depolarized, with a PD of +10 mV, normal streaming, and G_{rev}(60) of 295 Sm^{-2}, far exceeding G_s.

cell had a resting PD of −66.8 mV, and was in leak state with a relatively low G_s of 10 Sm^{-2} (Fig. 6*a* and *b;* ▲). When transferred to 3/4 SW, streaming stopped and the cell depolarized by 42 mV. G_{rev}(initial) increased eight-fold to 84.4 Sm^{-2} (Fig. 6*a* and *b;* ●). After 45 min G_{rev}(30) declined, to 5 Sm^{-2}, and the PD recovered to −49.6 mV, with the cell entering K^+ state (Fig. 6*a* and *b;* △). G_{rev} steadily declined until it was less than G_s. Thus heparinase treatment had no dramatic effects on SW cells which were already deficient in mucilage.

Histochemical Staining in Heparinased and Untreated *Lamprothamnium* Cells

Toluidine Blue and Alcian Blue at pH 1 are not vital stains. The staining process kills the cells, but leaves the walls and mucilage intact. The following results thus concern different cells from those in electrophysiology experiments. However, where possible, the cells were from the same collection batch. Table 1 summarizes the intensity of staining reactions with Toluidine Blue at low pH. Figure 7 shows the staining reactions with both Toluidine Blue and Alcian Blue at low pH.

Young cells from the apical region of 1/3 SW plants had no apparent extracellular mucilage when viewed with dark-field optics (Table 1). These cells exhibited patches of barely discernible metachromatic staining with Toluidine Blue (Table 1) and no staining with Alcian Blue at low pH. These cells apparently lack extracellular sulfated polysaccharide mucilage.

Figure 7 shows staining reactions of extracellular mucilage in SW cells; Alcian Blue staining is compared in SW and heparinased SW cells (Fig. 7*a* and *b*) and Toluidine Blue staining is compared in SW and heparinased SW cells (Fig. 7*c* and *d*). Untreated SW cells were intensely stained (Fig. 7*a* and *c*, corresponding to +++++ in Table 1) and heparinased SW cells were very weakly stained (Fig. 7*b* and *d*, corresponding to + in Table 1).

Basal SW cells had thick extracellular mucilage when reviewed with dark-field optics (Table 1). The mucilage stained with Alcian Blue at pH 1 (Fig. 7*a*) and heavily and metachromatically with Toluidine Blue at pH 1 (Fig. 7*c*) indicating the presence of sulfated polysaccharides (Mariani, Tolomio & Braghetta, 1985). The staining was not homogeneous and usually had a layered appearance (visible beneath the arrow in Fig. 7*a* and *c*). Examination of unstained cells with phase-contrast mi-

Table 3. Mean rate of change of cell PD, mean membrane PDs and cytoplasmic streaming during the time course of the hypotonic response

Cell type	Resting PD (mV)	Mean rate of change of PD (mV/min) 0−1 min	Mean rate of change of PD (mV/min) 1−2 min	Mean rate of change of PD (mV/min) 2–3 min	PD (5–10 min) (mV)	PD (30 min) (mV)	PD (60 min) (mV)
1/3 SW	−138 ± 0.2 (n = 5)	+26.7 ± 0.5 (n = 7) Streaming slows	+30.7 ± 0.8 (n = 7) Streaming stops	+39.2 ± 0.8 (n = 7) Streaming restarts	−43.8 ± 0.8 (n = 5)	−88.1 ± 0.8 (n = 5)	−103.3 ± 0.9 (n = 5)
SW	−88.2 ± 0.86 (n = 7)	+0.7 ± 0.4 (n = 6) No streaming change	−7.9 ± 0.7 (n = 6) No streaming change	+4.8 ± 0.6 (n = 6) No streaming change	−70.6 ± 0.9 (n = 7)	−69.4 ± 0.8 (n = 7)	−67.8 ± 0.8 (n = 7)
Heparinased SW	−99.1 ± 1.9 (n = 4)	+28 ± 1 (n = 4) No streaming change	−13.5 ± 0.6 (n = 4) No streaming change	+41.3 ± 1.1 (n = 4) Streaming slows	+3.6 ± 1.2 (n = 4) Streaming stops; streaming recovers ~10 min	−13.5 ± 1.4 (n = 4)	+3.8 ± 1.6 (n = 4)
SW, further osmotic step from 3/4 to 1/2 SW	−69.2 ± 0.6 (n = 3)	+33 ± 0.4 (n = 3) Streaming slows					

The mean rate of change in depolarization was calculated from chart recordings.
Estimates of E_K and E_{Cl}. E_K in SW is −74.1 mV and E_{Cl} is +28 mV in SW, assuming $K_i^+ = 290$ mM, $Cl_i^- = 470$ mM (Bisson & Kirst, 1980*b*), and $K_o^+ = 16.1$ mM, $Cl_o^- = 400$ mM. Initially, when hypotonic solution is added, and before ion fluxes commence, E_K becomes − 81.5 mV and E_{Cl} + 11.5 mV. E_K in 1/3 SW is close to −80 mV.

croscopy also revealed layering, and so this was not due to the staining procedure. The proportion of sulfated polysaccharides was estimated from photographs of stained cells to vary from ~75–~90%. The mucilage of SW cells remained resistant to degradation for weeks after a cell died.

Heparinased SW cells had extracellular mucilage of similar initial thickness (Table 1). This was still present, viewed with dark-field optics, after 18 hr heparinase treatment. If there was a change in the thickness due to heparinase treatment, it was not easily observable at the magnifications used. However, the layer had become more opaque. After 2.5 days the mucilage was visibly thicker and sloughed off in a loose opaque sleeve (Fig. 7*d*). An underlying mucilage layer was still visible at the interface with the cell wall suggesting that the layer is continuously synthesized. Heparinased SW cells stained only very faintly with Alcian Blue at pH 1 (Fig. 7*b*). The residual staining was confined to the interface between mucilage and cell wall. The heparinased cells either stained very faintly, giving a pink coloration (Fig. 7*d*) rather than a metachromatic reaction, or did not stain at all with Toluidine Blue. Overall the staining reaction for sulfated polysaccharides was lost after heparinase treatment (Table 1). The mucilage of heparinased cells was no longer resistant to degradation and after 3 days it was heavily colonised by bacteria and protozoa, despite the initially aseptic preparation.

Toluidine Blue staining of extracellular mucilage did occur when heparinased SW cells were returned to SW for 18 hr following 18 hr heparinase treatment (Table 1) although the extent of staining was at the lower end of the range found in controls (estimated as between ~50 and 60%).

Discussion

Extracellular Mucilages in Algae

In addition to the capacity to partially or completely regulate turgor, marine algae commonly display adaptations of the cell wall and extracellular matrix (ECM) which are thought to affect ionic relations. The ECM of marine algae frequently contains sulfated polysaccharide mucilages whereas the ECM of freshwater algae does not (Kirst, 1989). Scanning electron microscopic analysis showed that sulfate groups in algal mucilage bind cations such as Ca^{2+} (Mariani et al., 1990). The abundance of

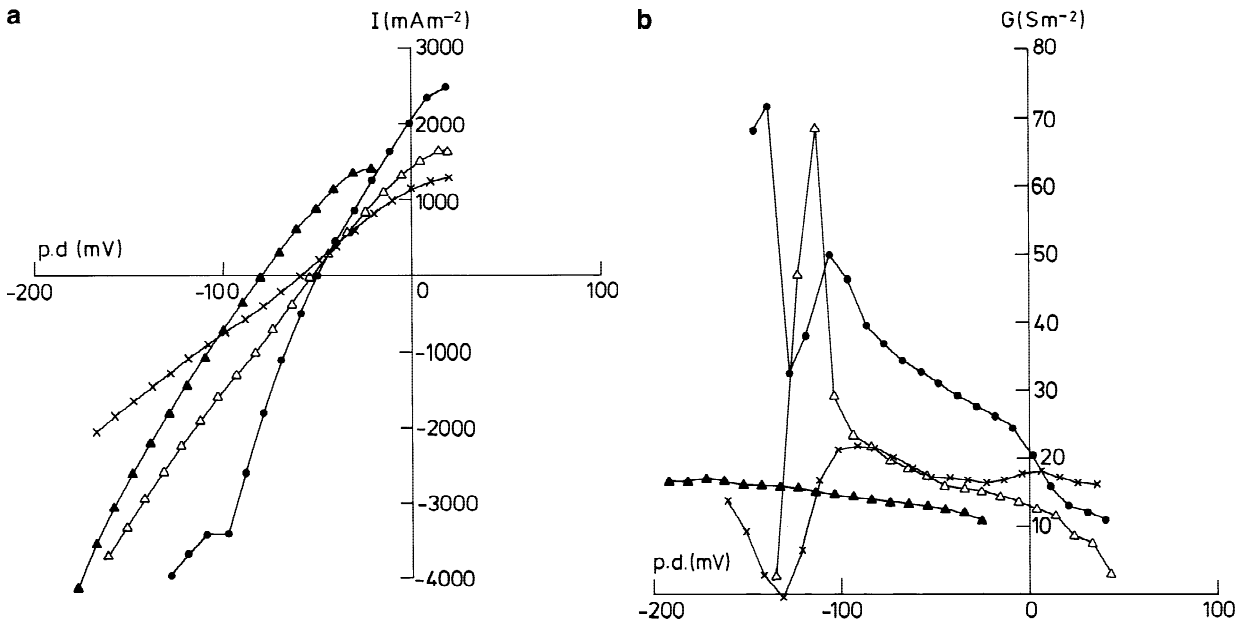

Fig. 5. *I/V* (*a*) and *G/V* (*b*) profiles show recovery of the normal hypotonic response, typical of SW cells, when heparinase was removed overnight. The figure shows the same cell as in Fig. 4, with heparinase washed out overnight, going from 3/4 to 1/2 SW. (▲). In 3/4 SW, the cell was relatively depolarized with a PD of −79.9 mV and with G_s of 34 Sm^{-2}. The cell had rapid cytoplasmic streaming. (●). On introduction of 1/2 SW the cell continued cytoplasmic streaming but depolarized to −44.9 mV. After 28 min in 1/2 SW, the cell had begun hyperpolarizing, to −49.80 mV, with G_{rev}(30) of 54 Sm^{-2}, and rapid streaming. The cell had entered the K^+ state. (△). After 63 min in 1/2 SW, G_{rev}(60) had declined to 33 Sm^{-2}, slightly less than G_s, with a PD of −53.9 mV. (x). After 99 min in 1/2 SW, G_{rev} had declined still further to 21 Sm^{-2}, less than G_s, and the cell had hyperpolarized to −60.9 mV. The K^+ state is still evident.

sulfate groups varies between species, reflecting ecophysiological roles (Mariani et al., 1990), and indeed, the polysaccharide composition can vary between young and mature plants, or different parts of the plant, or with season and environmental conditions (McCully, 1970). The eggs of *Fucus*, a brown marine alga, secrete a wall containing sulfate polysaccharides within minutes of fertilization, followed by secretion of another highly sulfated fucoidan within specialized Golgi-derived vesicles ("F-granules") ~8–10 hr post-fertilization (reviewed, Quatrano & Shaw, 1997). These F granules are responsible for polar deposition of cell wall material, which permanently establishes the asymmetry of the zygote. Secretion of a sulfated polysaccharide is thus critical for determining the subsequent body plan of the embryo. This must be associated with ion transport processes, since Ca^{2+} channels and localized changes in Ca^{2+} ion concentration at the rhizoid tip are also involved in establishment of polarity (Shaw & Quatrano, 1996; Quatrano & Shaw, 1997). Work with the F granules in *Fucus* suggests that the actin cytoskeleton could be linked to the ECM via an integrin-like protein (Shaw & Quatrano, 1996; Quatrano & Shaw, 1997).

Marine biologists accept that presence of abundant negatively charged polysaccharides, with their cation exchange properties, is one of the adaptations that allow seaweeds to colonize environments where ion concentration markedly fluctuate. These substances are also involved in development. Despite the important roles played by such highly charged polysaccharides in algae, there is still no direct evidence that they can impact upon ion transport, particularly transport of Ca^{2+}. Our results demonstrate that the extracellular sulfated polysaccharides of *Lamprothamnium* indeed influence cell electrophysiology in the resting state and during the response to osmotic shock.

Effect of Heparinase

Shepherd et al. (1999) described a progressive reduction in the magnitude of the hypotonic response (depolarization and conductance increase) with increasing thickness of the sulfated polysaccharide mucilage layer. Here we have compared two extremes- 1/3 SW cells lacking mucilage (Table 1) and SW cells thickly coated in sulfated polysaccharide mucilage (Table 1, Fig. 7*a* and *c*). Put in terms of Fig. 1, all three transporters were present in the hypotonic response of 1/3 SW cells, while transporter 1 was still observed, transporter 2 was not triggered and transporter 3 was diminished in the hypotonic response of SW cells. The *I/V* and *G/V* profiles of SW cells during the hypotonic response were similar to those of 1/3 SW cells treated with La^{3+} (Beilby & Shepherd, 1996), sug-

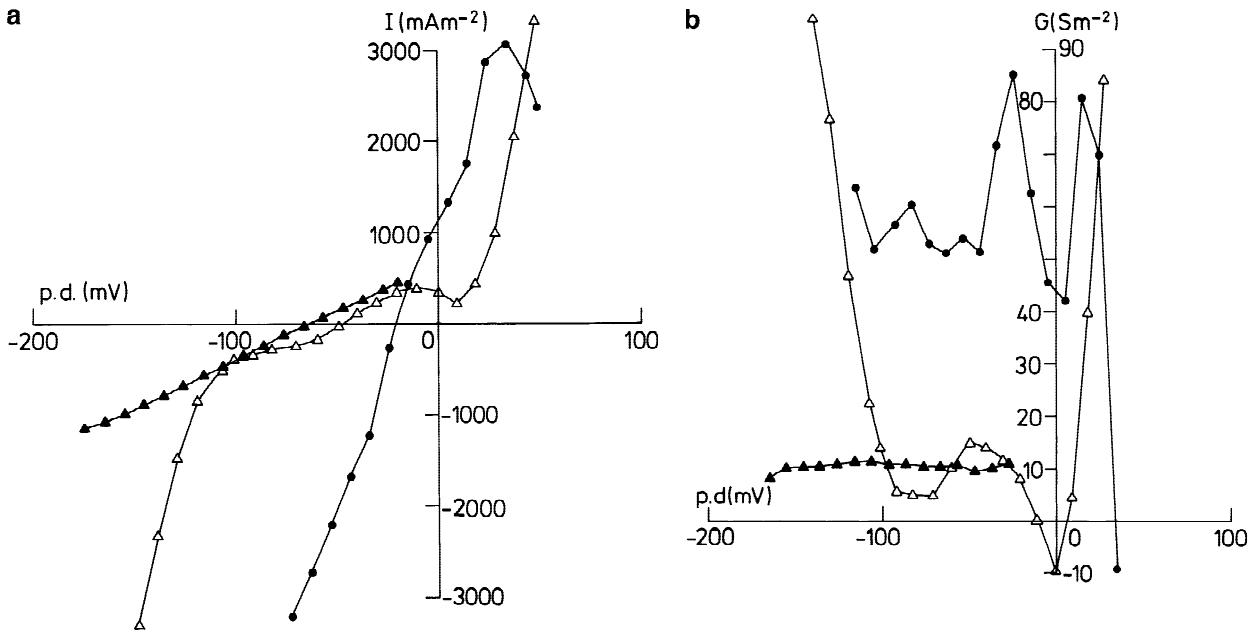

Fig. 6. *I/V* (*a*) and *G/V* (*b*) profiles of the response to hypotonic challenge (going from SW to 3/4 SW) of a heparinase-treated apical SW cell lacking sulphated polysaccharide mucilage (mucilage layer only 2.4 μm thick). (▲). In SW the cell had a resting PD of −66.8 mV, with close to linear profiles indicating the "leak" state. G_s was 10 Sm^{-2}. (●). After 3 min in 3/4 SW, cytoplasmic streaming had stopped and the cell had depolarized to −24.2 mV. G_{rev}(initial) increased over G_s, to 84.4 Sm^{-2}. (△). After 45 min in 3/4 SW, streaming had resumed (5 min after ceasing), and the cell had hyperpolarized to −49.6 mV, with G_{rev}(30) declining to 5 Sm^{-2}, less than G_s. Compare this to the very different behavior of the heparinased SW cell with thick mucilage (Fig. 4), and the behavior of the untreated mucilaginous SW cell (Fig. 3).

gesting that Ca^{2+} channels and Ca^{2+}-activated Cl^- channels were not activated. However, the high conductance Ca^{2+}-activated Cl^- and K^+ channels are indeed present and can be triggered in mucilaginous SW cells, but this requires a much larger osmotic step to 1/2 SW (Shepherd et al., 1999).

In the heparinased SW cells, the extracellular mucilage lost the capacity for binding the cationic dyes Toluidine and Alcian Blue at low pH (Table 1, Fig. 7*b* and *d*), but the 18-hr treatment did not remove the extracellular mucilage or cause obvious differences to its volume. Thus, the cation-exchange properties of the mucilage were lost, but it was still present as a substantial unstirred layer.

The Steady State

We have noted previously that G_s increases with increasing salinity of the medium (Shepherd et al., 1999). The 1/3 SW, SW and heparinased SW cells had progressively increasing G_s (Table 2). Impermeant ions (such as Na^+) can enable higher conductivity of permeant ions traveling through diffusion-limited ion channels by providing electrostatic screening at the mouth of the channel (Lauger, 1976), an effect which would become more pronounced as ionic concentration increases. The large difference between G_s in heparinased SW and untreated SW cells could be due to differences in electrostatic screening by the mucilage which, in the treated cells, had lost its ability to bind the cationic stains.

Delay in Initial Hypotonic Response of SW Cells

The heparinased cells showed a blend between the responses of mucilage-deficient 1/3 SW cells and mucilaginous SW cells. Heparinased SW cells rapidly depolarized in the first min (by ~103 mV), like the 1/3 SW cells, but transiently hyperpolarized at around 2 min, like the SW cells (Table 3). The source of this hyperpolarization is unknown, but it is possibly the proton pump, which can hyperpolarize the resting PD to levels more negative than −200 mV (Bisson & Kirst, 1980; Beilby & Shepherd, 1996). Experiments using inhibitors of the proton pump would be necessary to test this possibility. Reduction in turgor caused pump depolarization in *Chara* (Beilby & Walker, 1996) and it is possible that increase in turgor promotes transient pump hyperpolarization. The turgor increase was gradual in the SW and heparinased SW cells, since mucilaginous SW cells have a much lower hydraulic conductivity than cells deficient in mucilage (Shepherd et al., 1999). Clearly the heparinased mucilage still behaved as an unstirred layer which delayed the full impact of hypotonic shock, so that the cells did not immediately "see" the change in external

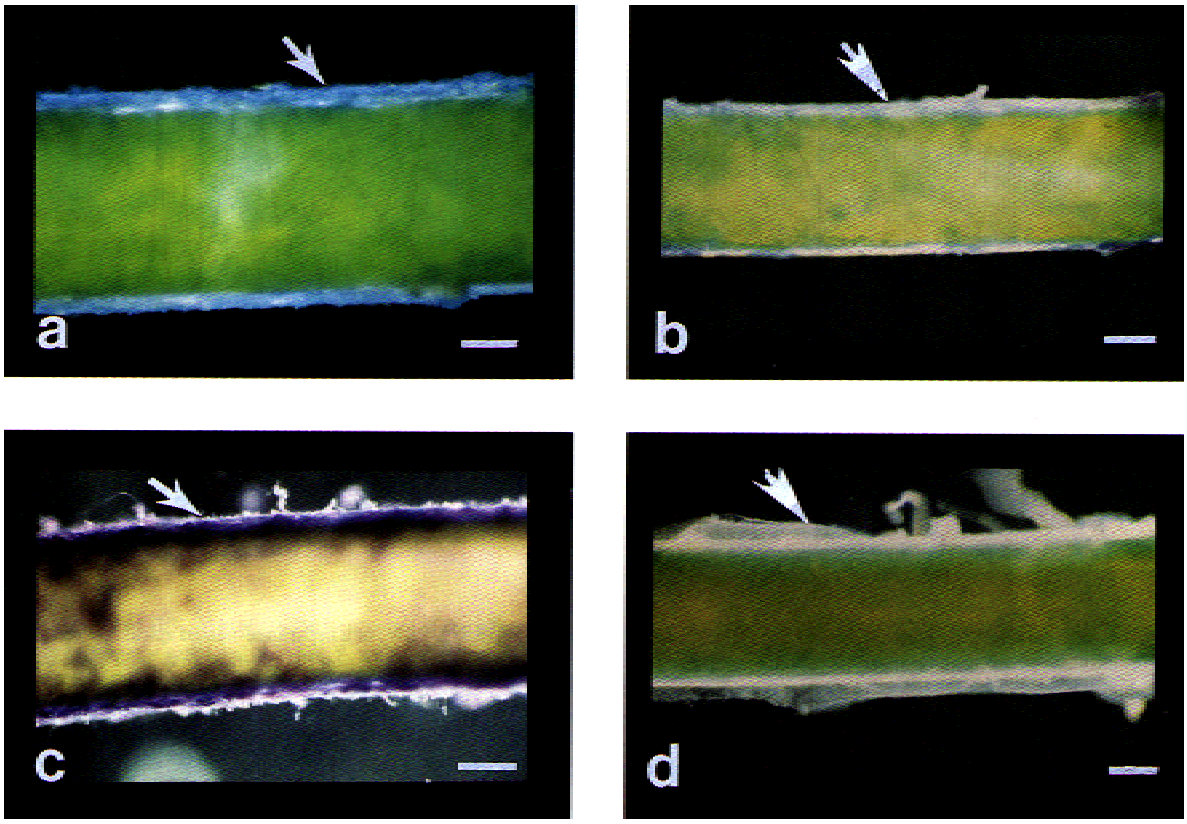

Fig. 7. Micrographs of heparinased and untreated SW *Lamprothamnium* cells with extracellular mucilage layers stained using Toluidine Blue or Alcian Blue at pH 1. At this low pH, only sulfated polysaccharides are stained. (*a*) Untreated basal SW cell stained with Alcian Blue at pH 1, showing the layered and stained appearance of the extracellular mucilage (arrow) indicating presence of sulphated polysaccharides. Bar = 50 μm. (*b*) Heparinased basal SW cell, stained with Alcian Blue at pH 1. The mucilage layer is only faintly stained, close to the cell wall (arrow), indicating disruption of the polyanionic structure, which however is not dissolved, and remains as a gel layer. Bar = 100 μm. (*c*) Untreated basal SW cell stained with Toluidine Blue at pH 1. The mucilage layer stains heavily (arrow), indicating presence of sulfated polysaccharides. Bar = 50 μm. (*d*) Heparinased basal SW cell stained with Toluidine Blue at pH 1, showing greatly reduced staining reaction for sulfated polysaccharides. This cell was treated with heparinase for 2.5 days, and the exterior of the mucilage layer had been sloughed off (arrow), leaving a new, but still scarcely stained layer beneath. The mucilage was attacked by protozoans and bacteria, although the mucilage of even dead but untreated SW cells remained resistant. Bar = 100 μm.

ion concentration. Under such conditions, the putative effect on the proton pump is discernible before the high conductance transporters (2 and 3 in Fig. 1) become engaged. This hypothesis will be tested by applying pump inhibitors as well as small hypotonic steps to both mucilaginous and mucilage-free cells.

The Hypotonic Response

The mucilage of heparinased cells remained in place, but no longer reacted with cationic stains at low pH (Fig. 7). Thus we can separate the effect of the mucilage as an unstirred layer from its polyanionic (Ca^{2+} in binding) properties. In heparinased SW cells the rapid depolarization proceeded after 3 min, when cytoplasmic streaming slowed and stopped, as occurred in 1/3 SW, but not SW cells (Table 3). The cells recovered other features of the acute response of nonmucilaginous 1/3 SW cells. The streaming cessation indicates opening of Ca^{2+} channels, and the subsequent conductance increase must be due to Ca^{2+}-activated Cl^- channels, with G_{rev} increasing to ~4 times G_s in the initial phase of the response (Table 2; Fig. 4). A similar large conductance increase occurred in the 1/3 SW, but not the SW cells. The increase in cytoplasmic Ca^{2+} ion concentration was transient, as in the 1/3 SW cells, because streaming was recovered (Table 3). The major difference between the early response of heparinased SW and SW cells is the recovery by heparinased cells of the Ca^{2+}-mediated part of the response.

While the recovery of the initial part of the hypotonic response in heparinased SW cells is explicable in terms of loss of cation-binding properties of the mucilage, the subsequent behavior of the heparinased cells is less easily explained. Unlike both 1/3 SW and SW cells,

heparinased SW cells did not restore membrane PD (which remained positive, in the vicinity of estimated E_{Cl}) and failed to restore conductance (Tables 2 and 3, Fig. 4). While the "shoulder" in the *I/V* and *G/V* profiles (Fig. 4) at 30 and 60 min suggests that K^+ channels opened, any indication of the K^+ state was swamped by the large conductance and positive PD. The failure of heparinased cells to recover PD or conductance might be due to "jamming open" of mechanism 1, with Cl^- effluxing through the putative SA channels, consistent with the very depolarized PD. However, pharmacological dissection would be necessary to test this possibility.

The full recovery of normal SW cell behavior when heparinase was removed (Fig. 5) suggests that the response of the heparinased SW cells was not due to nonspecific damage. Since heparinase removal resulted in partial restoration of the capacity of the mucilage to stain with cationic dyes at low pH (Table 1) the mucilage must be continuously synthesized, perhaps accounting for the banded appearance of mucilage in some control cells (Fig. 7*a* and *c*). Furthermore, the hypotonic response of apical SW cells (which were deficient in mucilage) was unaffected by heparinase treatment (Fig. 6). There must be other differences between the strategies of mucilaginous and nonmucilaginous cells for dealing with hypotonic shock. These probably involve differences in vacuolar structure and compartmentation processes (Beilby et al., 1999).

The response of 1/3 SW cells to hypotonic shock was similar to the hypotonic response of rhizoids in *Fucus.* Here a transient increase in cytoplasmic Ca^{2+} ion concentration, from nanomolar to micromolar concentration, occurred ~10 sec after the cell volume increased (Taylor et al., 1996).

In patch-clamp experiments on *Fucus* protoplasts Taylor et al. (1996) identified mechanosensory (stretch-activated) plasmalemma channels which carried K^+ outward and Ca^{2+} inward. Hypotonic shock induced Ca^{2+} ion influx, which was then propagated throughout the rhizoid with contributions from intracellular stores. Cytoplasmic Ca^{2+} ion concentration also increases during the hypotonic response of young small cells of salt-tolerant *Chara longifolia* (Bisson et al., 1995). It may be a generalized requirement for the acute hypotonic response in marine algal cells lacking extracellular sulfated polysaccharides.

The triggering of the transporter 2 (Fig. 1), exemplified by 1/3 SW cells, appears to depend on the rate of initial depolarization, which was significantly faster in 1/3 SW cells, heparinased SW cells (preceding streaming cessation), and SW cells given a further osmotic step from 3/4 to 1/2 SW within half an hour of the initial response (Table 3; *see also* Shepherd et al., 1999). The initial rate of membrane stretching as water enters could be reflected in the rate of depolarization, which would represent the collective behavior of stretch-activated channels (Fig. 1). These may "prime" the Ca^{2+} channels which cause cytoplasmic Ca^{2+} concentrations to transiently increase and which activate the Cl^- channels responsible for large conductances. The fast rate of depolarization appears to be inseparable from the triggering of Ca^{2+} channels and the opening of Cl^- channels bringing about large cell conductances (Tables 2 and 3).

Are stretch-activated (mechanosensory) channels likely to be found in the plasmalemma of *Lamprothamnium?* Such channels were found to interact with voltage dependent and highly conductive K^+ and Cl^- channels in bean guard cells (Cosgrove & Hedrich, 1991). In freshwater *Chara*, there are two kinds of stretch-activated (mechanoperceptive) channels, one of which is attached to the ECM (Staves & Wayne, 1993). Mechanosensory Ca^{2+} and Cl^- channels have been reported in *Chara* by Shimmen (1996, 1997); these are not inhibited by La^{3+}. Thus, there are precedents in charophytes and higher plants for mechanosensory channels responding to pressure changes and interacting with channels responsible for large whole-cell conductances. It is useful to identify a role for such channels in an actual life process of *Lamprothamnium* before applying more disruptive approaches, such as patch-clamping, to the transporters.

Similarities Between Animal and Algal Systems

Sulfated polysaccharide mucilages are found in red, green and brown algae. In the latter two groups they are referred to as heparinoids, emphasizing their close structural relationship to heparin, a sulfated polysaccharide of the animal kingdom, which is well-known for its anticoagulant properties (Maeda et al., 1991). There is considerable interest in industrial and medical applications of such mucilages, as blood anticoagulants (Maeda et al., 1991), as anti-HIV chemicals (Beress et al., 1993) and as gel stabilizers (Yaron, Cohen & Arad, 1992). These important properties may relate to the original role of these molecules in salinity tolerance. Observation of their role in vivo can only contribute to an understanding of their remarkable properties in vitro. Since it is disrupted by heparinase, we regard the *Lamprothamnium* mucilage as a heparinoid. Heparin has pronounced effects on electrophysiology of animal cells, where, for example, it alters the membrane conductance of erythrocytes (Markushin, Suyushev & Chernov, 1988) and modifies excitation/contraction coupling in animal cells through its effects on Ca^{2+} channels (Lamb, Posterino & Stephenson, 1994). In animal cells the heparin receptor is directly coupled to the Ca^{2+} channel (Knauss et al., 1992). Our study is the first to demonstrate a direct link between the presence of an extracellular heparinoid and electrophysiological behavior of an algal cell. It may well be that subsequent experiments will reveal further similarities with animal systems, where such molecules

are also associated with situations where rapid and unpredictable changes in osmolarity occur.

The acute hypotonic response of most cells living in saline environments, whether animal, plant or fungal, involves opening of Cl^- channels responsible for large conductances, and K^+ channels, leading to an efflux of K^+ and Cl^-, water loss, and restoration of volume. Compared with marine algae, mammalian cells are normally insulated against sudden changes in osmolarity. Nonetheless the acute response to hypotonic shock in bovine cells resembles fundamental aspects of the response of 1/3 SW *Lamprothamnium* cells. Following hypotonic shock, a swelling-activated (equivalent to plant "mechanosensory" or "stretch-activated") Cl^- channel initiates Cl^- efflux, depolarizing the membrane and opening voltage-gated K^+ channels, resulting in water loss and restoration of cell volume (Srinivas, Bonanno & Hughes, 1998). This resembles the opening of Cl^- channels, responsible for large conductances in the early stages of the response in 1/3 SW *Lamprothamnium* cells, which is followed by opening of K^+ channels, also responsible for large whole-cell conductance, resulting in the subsequent decrease in whole cell conductance seen in Fig. 2. Considering the wide evolutionary divergence between charophytes and cows, the turgor/volume regulatory mechanism must have evolved very early and remain conservative.

This research was funded by Australian Research Council Large Research Grant A19602591, which is gratefully acknowledged. We would like to thank Dr. G. Siegel for suggesting the use of heparinase.

References

Beilby, M.J. 1990. Current-voltage curves for plant membrane studies: A critical analysis of the method. *J. Exp. Bot.* **41:**165–182

Beilby, M.J., Cherry, C.A., Shepherd, V.A. 1999. Dual turgor regulation response to hypotonic stress in *Lamprothamnium papulosum. Plant Cell Environ.* **22:** 347–360

Beilby, M.J., Mimura, T., Shimmen, T. 1997. Perfusion of charophyte cells: a critical analysis of the method. *J. Exp. Bot.* **48:**157–172

Beilby, M.J., Shepherd, V.A. 1996. Turgor regulation in *Lamprothamnium papulosum* 1. *I/V* analysis and pharmacological dissection of the hypotonic effect. *Plant Cell Environ.* **19:**837–847

Beilby, M.J., Walker, N.A. 1996. Modeling the current-voltage characteristics of *Chara* membranes: I. The effect of ATP removal and zero turgor. *J. Membrane Biol.* **149:**89–101

Beress, A. Wasserman, O., Bruhn, T., Beress, L., Kraiselfburd, E.N., Gonzalez, L.V., Demotta, G.E., Chavez, P.I. 1993. A new procedure for the isolation of anti-HIV compounds, polysaccharides and polyphenols from the marine alga *Fucus vesiculosus. J. Natural Prod.* **56:**478–488

Bisson, M.A., Kiegle, E., Black, D., Kiyosawa, K., and Gerber, N. 1995. The role of calcium in turgor regulation in *Chara longifolia. Plant Cell Environ.* **18:**129–137

Bisson, M.A., Kirst, G.O. 1980*a*. *Lamprothamnium*, a euryhaline charophyte I. Time course of turgor regulation. *J. Exp. Bot.* **31:**1223–1235

Bisson, M.A., Kirst, G.O. (1980*b*). *Lamprothamnium*, a euryhaline charophyte II. Osmotic relations and membrane potential at steady state. *J. Exp. Bot.* **31:**1237–1244

Cosgrove, D.J., Hedrich, R. 1991. Stretch-activated chloride, potassium and calcium channels coexisting in plasma-membrane of guard cells of *Vicia faba. Planta* **186:**143–153

Kirst, G.O. 1989. Salinity tolerance of eucaryotic marine algae. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **40:**21–53

Knauss, H.-G., Moshammer, T., Friedrich, K., Kang, H.C., Haugland, R.P., Glossmann, H. 1992. In vivo labeling of L-type Ca^{2+} channels by fluorescent dihydropyridines: Evidence for a functional, extracellular heparin-binding site. *Proc. Natl. Acad. Sci. USA* **89:**3586–3590

Lamb, G.D., Posterino, G.S., and Stephenson, D.G. 1994. Effects of heparin on excitation-contraction coupling in skeletal muscle of toad and rat. *J. Physiol.* **474:**319–329

Lauger, P. 1976. Diffusion-limited ion flow through pores. *Biochim. Biophys. Acta* **455:**493–509

Maeda, M., Uehara, T., Harada, N., Sekiguchi, M., Hiraoka, A. 1991. Heparinoid-active sulphated polysaccharides from *Monostroma nitidum* and their distribution in the Chlorophyta. *Phytochem.* **30:**3611–3614

Mariani, P., Tolomio, C., Baldan, B., Braghetta, P. 1990. Cell wall ultrastructure and cation localisation in some benthic marine algae. *Phycologia* **29:**253–262

Mariani, P., Tolomio, C., Braghetta, P. 1985. An ultrastructural approach to the adaptive role of the cell wall in the intertidal alga *Fucus virsoides. Protoplasma* **128:**208–217

Markushin, Y.Y., Suyushev, V.A., Chernov, Y.P. Change in membrane conductivity of erythocytes in the presence of heparin. *Biophysics* **33:**562–564

McCully, M.E. 1970. The histological localization of the structural polysaccharides of seaweeds. *Ann. N.Y. Acad. Sci.* **175:**702–711

O'Brien, T.P., McCully, M.E. 1981. The Study of Plant Structure: Principles and Selected Methods. Termarcarphi Pty Ltd. Melbourne, Australia

Quatrano, R.S., Shaw, S.L. 1997. Role of the cell wall in the determination of cell polarity and the plane of cell division in *Fucus* embryos. *Trends Plant Sci.* **2:**15–21

Shaw, S.L., Quatrano, R.S. 1996. The role of targeted secretion in the establishment of cell polarity and the orientation of the division plane in *Fucus* zygotes. *Development* **122:**2623–2630

Shepherd, V.A., Beilby, M.J., Heslop, D.J. 1999. Ecophysiology of the hypotonic response in the salt-tolerant alga *Lamprothamnium papulosum. Plant Cell Environ.* **22:** 333–346

Shimmen, T. 1996. Studies on mechano-perception in characean cells: development of a monitoring apparatus. *Plant Cell Physiol* **37:**591–597

Shimmen, T. 1997. Studies on mechanoperception in Characean cells: pharmacological analysis. *Plant Cell Physiol.* **38:**139–148

Srinivas, S.P., Bonanno, J.A., Hughes, B.A. 1998. Assessment of swelling-activated Cl^- channels using the halide-sensitive fluorescent indicator 6-methoxy-N-(3-Sulfopropyl) quinolinium. *Biophys. J.* **75:**115–123

Staves, M.P., Wayne, R. 1993. The touch-induced action potential in *Chara:* inquiry into the ionic basis and the mechanoreceptor. *Aust. J. Plant Physiol.* **20:**471–488

Taylor, A.R., Manison, N.F.H., Fernandez, C., Wood, J., Brownlee, C. 1996. Spatial organization of calcium signaling involved in cell volume control in the *Fucus* rhizoid. *The Plant Cell* **8:**2015–2031

Yaron, A., Cohen, E., Arad, S. 1992. Stabilization of *Aloe vera* gel by interaction with sulfated polysaccharides from red microalgae and with xanthan gum. *J. Agric. Food Chem.* **40:**1316–1320