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Measurement of the Sieve Tube Membrane Potential[1,2]

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ABSTRACT

A procedure is described for the measurement of the sieve tube membrane potential in the phloem of bark strips from *Salix exigua* Nutt. Measurements were made by inserting a measuring microelectrode into sap exuding from severed stylets of the willow aphid, *Tuberolachnus salignus*. Data taken from 20 bark strips gave an average potential of −155 ± 9 millivolts. Evidence is presented for an electrogenic component of the sieve tube membrane potential. The occurrence of a saturable sucrose-induced membrane depolarization is consistent with the concept of sugar accumulation by a sucrose/H^+ co-transport mechanism.

Recent work on the accumulation of sugars by the phloem has implicated the role of a H^+/sugar co-transport mechanism as the energetic basis for active sugar transport into the sieve tubes. Aside from the analogy to the better characterized systems in bacteria and fungi, this concept receives firm support from the sucrose-induced alkalination of the medium during uptake by *Ricinus* phloem (9) and by sugar beet phloem (3). Fusicoccin, a substance known to hyperpolarize the membrane potential, stimulates sugar uptake into the phloem (3, 10), whereas increased potassium concentration decreases uptake, due presumably to decreased membrane potential (2, 7). Sucrose-induced H^+-uptake into the phloem is strongly inhibited by respiratory inhibitors such as FCCP[3] (3, 6) and CCCP (9).

Although the available data, along with the marked H^+ gradient between the sieve tube contents and the apoplast (2), strongly support the concept that the protonmotive force provides the energy for sucrose accumulation into sieve tubes, there are no reliable measurements of the sieve tube membrane potential, which also would contribute to the proton motive force. Using [^{14}C]tetraphenylphosphonium, Komor (6) and Komor *et al.* (7) measured an "average" membrane potential in *Ricinus* cotyledons of −120 mv but, as the authors realize, this does not necessarily reflect the sieve tube membrane potential, nor does the procedure allow the detection of transient sucrose-induced depolarization. Bowling (1) measured putative sieve tube membrane potentials in hand sections of *Vitis* phloem but found only slightly negative (about −20 mv), or even positive, potentials.

This paper describes procedures for the use of severed aphid stylets to measure the sieve tube membrane potential in *Salix exigua* phloem. Evidence is presented that part of the membrane potential is electrogenic, and that it is depolarized by exogenous sucrose in a manner consistent with a sucrose/H^+ co-transport mechanism of uptake.

MATERIALS AND METHODS

Strips of bark approximately 1 cm × 7 cm × 1.0 mm thick were removed from 3- to 5-year-old willow stems (*S. exigua* Nutt.) and sealed into Plexiglas holders using a lanolin:paraffin mixture (1:4). The cambial surface was constantly irrigated with Higinbotham's 1 × nutrient solution (4) composed of 1 mM KCl, 1 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$, 0.904 mM NaH_2PO_4, and 0.048 mM Na_2HPO_4 at pH 5.7. Ten to 15 willow aphids (*Tuberolachnus salignus* Gmelin) were placed on a bark strip and allowed to settle overnight, following which they were anesthetized with CO_2 and their stylets were cut with a small razor blade fragment. The labium was brushed away from a selected stylet, and the remaining stylets were pulled from the bark with forceps.

For measurements of sieve tube membrane potential, a measuring electrode was placed in contact with a droplet of sap exuding from a severed stylet and a reference (ground) electrode was placed in the solution bathing the cambial surface. It was often necessary to position a capillary tube near the stylet to remove excess exudate from around the electrode, preventing exudate from contacting the surface of the bark. For K^+ concentration measurements, an ion-selective microelectrode was placed in contact with the exudate droplet along with the previously positioned measuring electrode which, for purposes of K^+ measurement, was grounded for use as a reference electrode. The electrodes were placed in such a manner that the stylet exudate flowed first past the potassium electrode, then past the reference electrode and into the collecting capillary. To prevent evaporation from the exudate, the electrode tips, stylet, and a portion of the bark surface were enclosed in a moist chamber. Voltage measurements were made with a Keithley 610B electrometer (10^{14}-ohm input impedance) and recorded on a strip-chart recorder. The bark strip, electrodes, leads, and a dissecting microscope were all enclosed in a grounded Faraday cage.

Reference and measuring microelectrodes were made from micropipettes pulled from melting point capillary tubing (Corning No. 9630) and were filled under vacuum with 3 M KCl at pH 4.0. Just prior to use, the tips were broken to a diameter of 50 to 100 μm and the micropipettes were refilled with 3 M KCl in 2% agar. Ag/AgCl electrodes were inserted into the agar.

Potassium ion-selective electrodes were made by siliconizing inside the tip of a micropipette followed by filling with K^+-selective liquid ion-exchange resin (Corning No. 477317). The remainder of the electrode was filled with 3 M KCl and a Ag/AgCl electrode (12).

During an experiment the cambial surface was constantly irrigated with a dilute nutrient solution, displacing the solution under the bark at a rate of six times/min, thus allowing for rapid changes of solution.

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[2] This paper is dedicated to the memory of Dr. Noe Higinbotham, who provided his criticisms and valued encouragement during the formative stages of our measurements of the sieve tube membrane potential.

[3] Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; E_m, measured membrane potential; E_k, Nernst potential for potassium.

In experiments using sugars at different concentrations, the sugars were dissolved in the 1 × nutrient solution and enough mannitol was added to maintain a constant osmolality. Potassium ions were varied as K_2SO_4 in a solution of 1 mM $Ca(NO_3)_2$, 0.25 mM $MgCl_2$ and 1 mM Mes buffer adjusted to pH 5.6 with Tris base. All inhibitors were dissolved in 1 × nutrients except FCCP, which was dissolved in the solutions used to vary potassium. All sugars, sugar analogs, FCCP, and DNP were purchased from Sigma; Na_3VO_4 was purchased from Fisher Scientific.

Except for those experiments on light/dark transitions, all measurements were made while the bark strip was illuminated by white light from a microscope illuminator. All experiments were repeated at least three times.

RESULTS

Our first attempts to measure sieve tube membrane potentials using severed aphid stylets were on an intact willow stem where the measuring electrode was placed in the stylet exudate droplet and the reference electrode was placed in a dilute salt solution on an abraded portion of the bark. Our first three attempts gave values of −118, −125, and −130 mv. Subsequent experiments employed bark strips as described under "Materials and Methods," as this allowed more precise control of the external solution. The average potential measured from 20 bark strips was −155 ± 9 mv when a 1 mM KCl solution bathed the cambial surface. This potential often remained nearly constant for several h under constant conditions. In these 20 bark strips, the highest potential recorded was −180 mv and the lowest was −123 mv. Spontaneous changes in flow rate from a single stylet (*e.g.* from no visible exudation to about 1 $\mu l\ h^{-1}$) did not affect the potential. Pulling the stylet partially from the bark caused exudation to stop and caused the potential to change markedly and unpredictably to unstable values, sometimes giving a positive reading on the electrometer. When stylet exudate contacted the bark strip, the recorded potential dropped to close to zero after several min unstable readings.

Application of KCN (Fig. 1) caused a rapid depolarization of the membrane potential. The potential slowly recovered to near its original value within 1 h after removal of the KCN (data not shown). In one experiment, recovery was almost complete within 15 min. Similar rapid depolarization of the membrane potential occurred on application of 20 μM FCCP, 2 mM DNP, or 10 mM Na_3VO_4. Recovery after removal of FCCP or DNP was much slower than for KCN and not as complete. Recovery from Na_3VO_4 was not followed.

Addition of K^+ to the solution bathing the cambial surface gave a rapid, reversible, and concentration-dependent depolarization (Fig. 2) that was not saturable even when the potential was made positive by continually increasing the K^+ concentration (data not shown). In one experiment (Fig. 3), the potassium concentration of the exudate was 65 mM as measured by the ion electrode. Addition of 20 μM FCCP (previously shown to give a maximal depolarization) caused the potential to change to −77 mv. At low external K^+ concentrations the measured E_m was less negative than the calculated potassium diffusion potential (E_k), whereas, at higher external K^+ concentrations the E_m was more negative than E_k. The shape of the external K^+ concentration *versus* E_m curve in the presence of uncoupler was little changed from the uninhibited tissue. In the absence of inhibitors, periodic measurements of exudate K^+ concentrations during the course of several experiments in which external K^+ or sucrose was varied showed little change (*i.e.* less than ±10%) in K^+ concentration during the several h required to run the experiments. Such measurements could not be made in the presence of FCCP because exudation was strongly inhibited.

Addition of sucrose to the bathing solution caused a rapid depolarization (Fig. 4) followed by a slow repolarization. The depolarization was saturated at higher sucrose concentrations. Removal of sucrose caused a rapid repolarization to a value several mv (in this case, 18 mv) more negative than the original potential, followed by a slow return to the original potential.

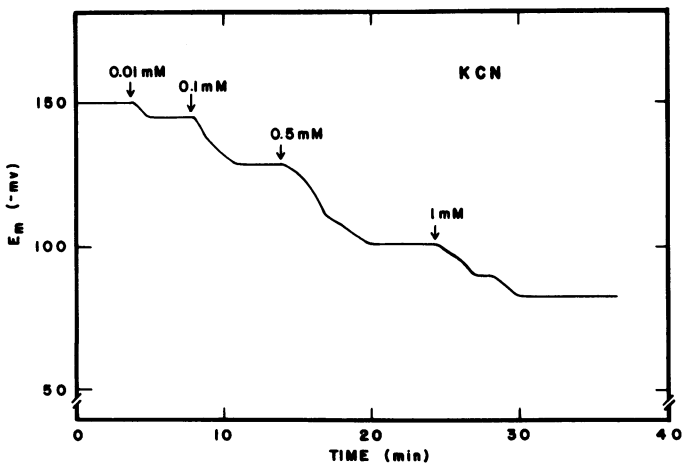

FIG. 1. Recorder trace showing the effect of cyanide on the sieve tube membrane potential.

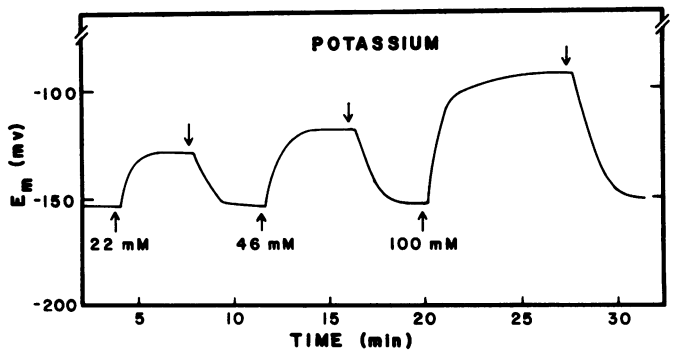

FIG. 2. Recorder trace showing the effect of potassium ions on the sieve tube membrane potential.

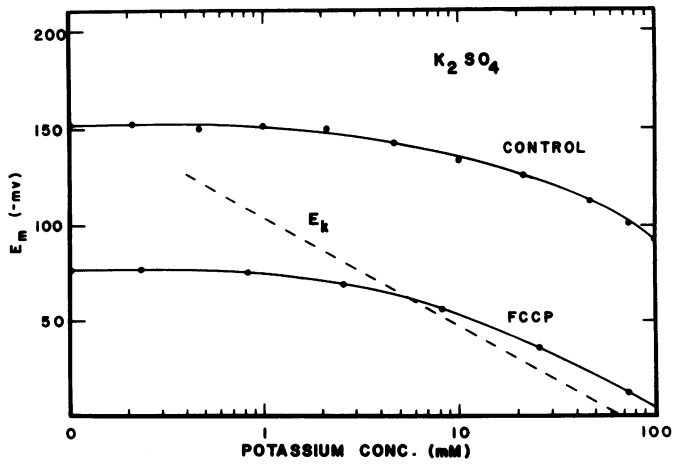

FIG. 3. The effect of increasing potassium ion concentrations on the sieve tube membrane potential before (control) and after adding 20 μM FCCP. Dashed line (E_k) gives the potassium diffusion potential assuming an internal concentration of 65 mM.

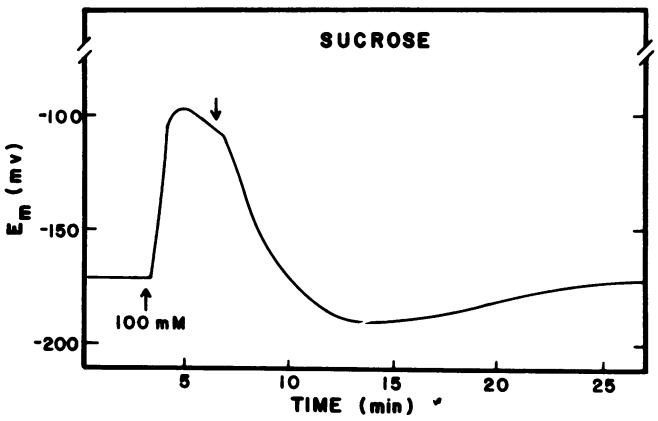

FIG. 4. Recorder trace showing the time course for the effect of 100 mM sucrose on the sieve tube membrane potential. The sucrose solution was introduced at the first arrow, followed by a solution of 100 mM mannitol, without sucrose, at the second (unlabeled) arrow.

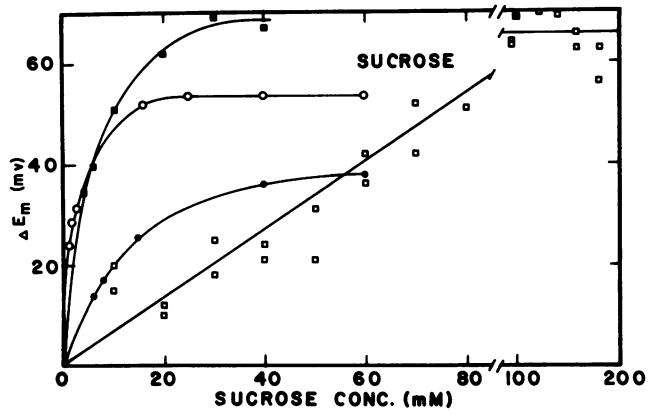

FIG. 5. Dependence of the sucrose-induced depolarization of the sieve tube membrane potential on sucrose concentration in experiments with four bark strips.

When sucrose was given at a subsaturating concentration, the potential could repolarize to near the original potential even in the continued presence of the sugar. Subsequent removal of sucrose then caused a hyperpolarization. Figure 5 shows the results from four experiments where sucrose solutions of varying concentrations were applied to the cambial surface. Values for half-maximal depolarization varied from approximately 2 to 50 mM, depending on the particular experiment.

To investigate the specificity of the sugar-induced depolarizations, a number of sugars and sugar analogs were applied, and the resulting depolarizations were compared to that caused by the same sucrose concentration. Mannitol gave no depolarization even at 150 mM. Sorbitol, 1-*O*-methylglucose, ribose, arabinose, and L-glucose had little effect, giving only 20 to 30% the response of sucrose. Fructose, D-glucose, 3-*O*-methylglucose, and raffinose were moderately effective at 50 to 70%; mannose was very effective, giving 90 to 100% the response of sucrose.

An additional response noticed was the effect of light on the sieve tube membrane potential. We consistently noticed that turning the lights off immediately initiated a hyperpolarization of 10 to 15 mv that reached a maximum in 5 min, followed by a slower depolarization to the original potential. Turning the lights on caused the opposite effect, *i.e.* a rapid depolarization followed by a slow repolarization. This was not an effect of light on the Ag/AgCl electrode because darkening the electrode changed neither the magnitude of nor the light/dark variations in the potential. Also, this was not an effect of temperature because filtering the light through water or using fiber optics gave the same response.

DISCUSSION

The successful use of aphid stylets to measure the sieve tube membrane potential depends on their functioning as an effective salt bridge between the sieve tube contents and the measuring electrode. Sieve tube exudates typically contain high potassium concentrations; our measurements of *S. exigua* exudates gave values ranging from 65 to 120 mM. A rough estimate of the stylet's expected electrical resistance can be calculated from the stylet food canal dimensions, which taper from 1.1 to 11 μm^2 over a distance of about 1.8 mm (11). Using an average area of 6 μm^2 and assuming the canal to be filled with 100 mM KCl, its resistance would be about 2.6×10^9 ohms. Although this is about 3 orders of magnitude greater than the typical resistance of a glass microelectrode, it is still well within the measuring abilities of the electrometer used, which has an input impedance of 10^{14} ohms.

All of our observations have been consistent with the presumption that we were, in fact, measuring the sieve tube membrane potential. The magnitude of the potential was typical of those observed for other higher plant cells and the characteristics of the potential's response to added inhibitors, salts and sugars, and to light/dark transitions are similar to other observations with plant cells (8). That the potential was generated by conditions at the tip of the stylet was indicated by the marked effect on the potential when the stylet was slightly disturbed and by the "shorting out" of the potential when stylet exudate touched the bark surface. The potential was not generated in some way by flow through the stylet (*e.g.* as a streaming potential) inasmuch as it remained constant during marked, but spontaneous, changes in the flow rate, probably caused by partial plugging of the food canal.

In common with most other plant cells, there is an electrogenic component to the sieve tube membrane potential. Its magnitude was substantially greater than that predicted for a diffusion potential, at least for potassium, and it seems very unlikely that any other cation would be present in sufficient concentrations to account for the high membrane potential. The expected effects of anion and H^+ gradients would tend to be towards a positive diffusion potential. Respiratory inhibitors rapidly depolarized the membrane potential to values comparable to the expected diffusion potential for potassium.

The rapid sucrose-induced depolarization of the membrane potential is consistent with an electrogenic co-transport of H^+ and sucrose into the sieve tube, as suggested by others (2, 5, 7, 9). The saturation of the depolarization at higher concentrations indicates a carrier-mediated process, but the concentrations of sucrose giving half-saturation of the depolarization varied, perhaps due to differences in the diffusion pathway between the sieve tube and external solution (13). If this interpretation is correct, the expected K_m for a sucrose carrier would be 5 mM or less.

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