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SUCROSE–PROTON EFFLUX FROM MAIZE SCUTELLUM CELLS*

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; sucrose–proton cotransport.

Abstract—Sucrose–proton efflux was studied using maize scutellum slices treated with mannose, an energy poison in maize scutellum cells. High rates of sucrose efflux required a pH of at least 8 and a penetrating cation in the bathing solution. A lipid-soluble cation, triphenylmethylphosphonium ion ($TPMP^+$), or Na^+ ions in the presence of a lipid-soluble anion, tetraphenylborate, could serve as penetrating cations. It was concluded that these cations were necessary to maintain electrical neutrality during sucrose–proton efflux either by entering the cell to compensate for the loss of protons ($TPMP^+$ or Na^+) or by facilitating anion efflux across the plasmalemma ($TPMP^+$). As a result of sucrose efflux, there was a 4-fold accumulation of $TPMP^+$ in the tissue, indicating a Nernst potential of 36 mV, negative inside, and showing that sucrose–proton efflux is electrogenic. Proton efflux, during sucrose efflux in the presence of $TPMP^+$, was measured by titration with alkali. By varying the experimental conditions, a range of values for sucrose and proton efflux was obtained. In all cases, the proton–sucrose stoichiometry was close to 1.0.

INTRODUCTION

Sucrose–proton cotransport (symport) has been proposed as the mechanism for sucrose transport across the plasmalemma of the maize scutellum cell [1]. Two kinds of evidence support this proposal. First, the addition of sucrose to the solution bathing scutellum slices causes a transient alkalinization of the bathing solution [1]. Second, sucrose efflux is accompanied by cation influx, and, therefore, is considered to be electrogenic [1–3]. In energy-poisoned scutellum cells, efflux of previously accumulated sucrose can drive Rb^+ influx [3]. This evidence suggests that sucrose is transported across the scutellum plasmalemma with a proton but without a compensating anion. However, in contrast to sucrose transport, quantitative data on proton transport are lacking for the scutellum cotransport system as well as for sugar transport systems in higher plants generally. The proton transported–sugar transported stoichiometry is not known for any sugar–proton cotransport system in higher plants, and this ratio may be directly related to the capacity of the transport system for sugar accumulation.

The usual method for determining stoichiometry is to measure the rate of increase of pH upon addition of [^{14}C]sucrose to the solution bathing the plant tissue [4]. This method could not be used with maize scutellum slices because during incubation the slices cause a continual decline in bathing solution pH; therefore, the small, transient rise in pH when sucrose is added to the bathing solution [1] is not a true measure of proton influx. For the experiments of this paper, a different method was devised based on measurement of proton and sucrose efflux from energy-poisoned scutellum slices. This paper deals first with the effects of lipid-soluble ions on sucrose efflux. Then it is shown that sucrose–proton effux is electrogenic and has a stoichiometry of one proton per sucrose molecule.

RESULTS AND DISCUSSION

Sucrose efflux in the presence of triphenylmethylphosphonium bromide

Sucrose efflux from scutellum slices requires a high pH and a penetrating cation in the bathing solution [3]. Presumably, the penetrating cation is necessary to compensate for the proton which is extruded along with sucrose during efflux. Efflux is enhanced in the presence of an energy poison that inhibits proton extrusion via the plasmalemma-bound ATPase, and, of course, it is necessary to inhibit this ATPase when proton–sucrose stoichiometry is to be determined. In the experiments of this section, triphenylmethylphosphonium ion ($TPMP^+$) was used as a compensating cation and mannose was used as an energy poison. $TPMP^+$ is a lipid-soluble cation that readily penetrates membranes, and its accumulation in cells (from μM solutions) has been used to measure membrane potentials [4, 5]. At the concentrations (mM) used in these efflux experiments, however, $TPMP^+$ should act as an energy poison along with mannose. Mannose causes a drastic reduction in ATP content of scutellum slices without increasing ADP [6], and, presumably, it is because of this that mannose inhibits the plasmalemma proton pump [7] and the utilization of endogenous sucrose [8]. Mannose and $TPMP^+$ also cause stored (vacuolar) sucrose to be released into the cytoplasm, where it is available for efflux across the plasmalemma [3].

For sucrose efflux experiments, scutellum slices were incubated in fructose for 2 hr (to increase their sucrose content to 60–70 μmol/0.5 g), in buffered mannose for 1

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hr, and finally, in buffered mannose plus $TPMP^+$ for 1–3 hr. Sucrose efflux was measured during the mannose and mannose plus $TPMP^+$ incubations.

Low rates of efflux were obtained at pH 7 and below in the presence or absence of $TPMP^+$ (Fig. 1). At pH 8, a low efflux rate was obtained in the absence of $TPMP^+$, but the rate greatly increased (about 15-fold) upon addition of $TPMP^+$. At pH 7.5, in the presence of $TPMP^+$, the sucrose efflux rate was only about twice that at pH 7 (data not shown), so efflux rate increased abruptly between pH 7.5 and 8.

The effect of pH does not appear to be on $TPMP^+$ penetration into the cells since $TPMP^+$ strongly inhibited sucrose synthesis over the entire pH range, 4–8 (Table 1). In the range 4–7, $TPMP^+$ caused a 65% inhibition of sucrose synthesis and at pH 8 the inhibition increased to 76%. In contrast, only low rates of sucrose efflux occurred in the pH range 4–7, but $TPMP^+$ caused a high rate of efflux at pH 8. Mannose was not used in the sucrose synthesis experiments, and the inhibition of sucrose synthesis and the increase in sucrose efflux were caused by $TPMP^+$ (Table 1).

The necessity for high pH suggests that sucrose–proton efflux requires a proton gradient, high inside. The cytoplasmic pH of mannose-treated scutellum slices has been estimated to be 7.5 (from the distribution of weak acids between tissue and bathing solution; unpublished results) so raising the pH of the bathing solution to 8 would create a proton gradient of the correct polarity. On thermodynamic grounds, the sucrose gradient should be steep enough to drive sucrose–proton efflux against a proton gradient of 1–2 pH units; therefore, kinetic factors may be responsible for the requirement of a high bathing solution pH. The electrical potential across the plasmalemma also must be considered because it is the chemical potential gradient of protons, not the concentration gradient, that drives sucrose–proton efflux [9]. However, $TPMP^+$ should prevent the development of large electrical potential differences (see below).

Rates of sucrose efflux at pH 8 increased as the concentration of $TPMP^+$ was increased from 2 mM to 20 mM (Fig. 2), indicating that penetration of $TPMP^+$ was limiting the rate of sucrose efflux. However, during extended incubation periods, sucrose efflux declined to a constant rate that was about the same in both 10 mM and 20 mM $TPMP^+$ (Fig. 3). The constant rate of sucrose efflux was obtained from slices whose sucrose content varied from 43 to 18 μmol (in Fig. 3, the slices contained 70 μmol sucrose at zero time). These results indicate that efflux rate is a function of $TPMP^+$ concentration and at least one other factor which is not tissue sucrose concentration.

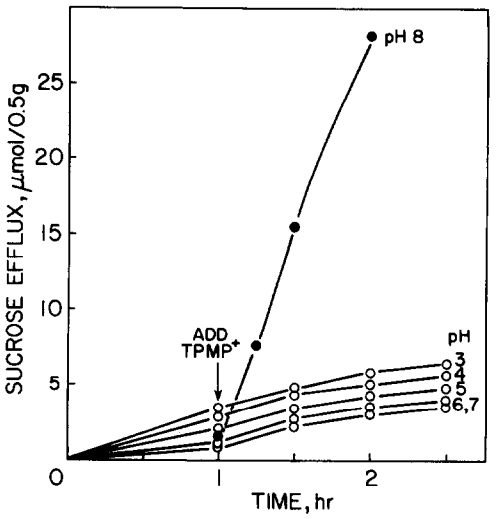

Fig. 1. Effect of pH on sucrose efflux. $TPMP^+$ concentration was 15 mM. Data are averages from 2 experiments.

The other factor limiting efflux could be the cellular concentration of mobile anions whose exit from the cell may be necessary to maintain electrical neutrality during sucrose–proton efflux. Possibilities for the maintenance of electrical neutrality during sucrose–proton efflux include: (a) $TPMP^+$ ions exchange for protons and remain in the cells. Accumulation of $TPMP^+$ to concentrations greater than the bathing solution concentration is supported by a membrane electrical potential, negative inside. (b) $TPMP^+$ quickly equilibrates across the plasmalemma by exchanging for protons as in the first possibility, above, or by entering the cell with an anion (e.g. Br^-). When equilibrium is reached, $TPMP^+$ entering the cell in exchange for a proton is balanced by $TPMP^+$ leaving the

Table 1. $TPMP^+$ inhibition of sucrose synthesis*

Bathing soln pH	$TPMP^+$ (20 mM)	Sucrose increase (μmol) Slices + Bathing soln = Total	Inhibition (%)
4	–	32.5 + 7.3 = 39.8 ± 2.0	
4	+	8.4 + 5.2 = 13.6 ± 1.4	66
5	–	30.2 + 9.4 = 39.6 ± 1.2	
5	+	5.7 + 8.6 = 14.3 ± 0.4	64
6	–	32.5 + 4.8 = 37.3 ± 1.5	
6	+	6.9 + 7.0 = 13.9 ± 0.0	65
7	–	30.4 + 4.5 = 34.9 ± 1.4	
7	+	6.9 + 6.4 = 13.3 ± 0.6	65
8	–	27.4 + 4.9 = 32.3 ± 1.2	
8	+	−19.2 + 27.1 = 7.9 ± 0.4	76

* Initially, the slices contained 31.6 + 0.5 μmol sucrose/0.5 g. The data are averages from 3 expts.

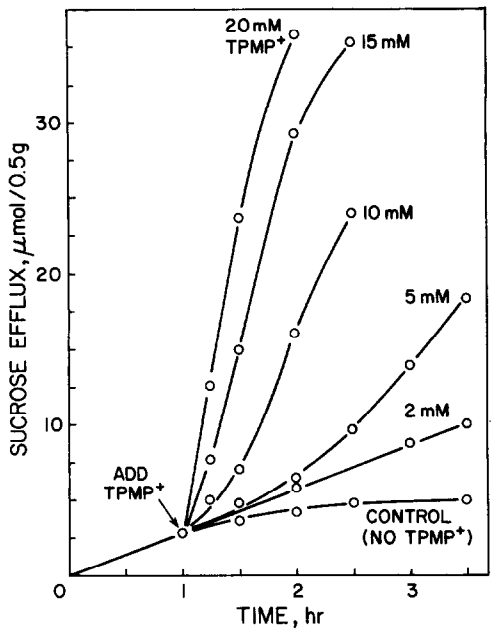

Fig. 2. Effect of $TPMP^+$ concentration on sucrose efflux. Bathing solution pH was 8.

cell complexed with an anion. (c) $TPMP^+$ ions in the membrane act as porters, moving anions across the plasmalemma to the cell exterior in accordance with the difference in anion concentration and electrical potential across the plasmalemma. In this mode of action, $TPMP^+$ is not a compensating cation but is a porter facilitating the exit of compensating anions. These possibilities could occur simultaneously, but if the membrane potentials created by sucrose–proton efflux are small (see below), electrical neutrality must be maintained to a large extent by anion efflux. It is suggested, therefore, that rates of sucrose efflux in Figs. 2 and 3 reflect cellular concentration of mobile anions. Initially the anion concentration was high, rates of sucrose efflux were high and $TPMP^+$ concentration was limiting efflux; later in the efflux period, anion concentration decreased and became limiting. The constant rate of sucrose efflux (Fig. 3) perhaps reflects the rate of anion production in the cell or the rate at which anions entered the cytoplasm from the vacuoles.

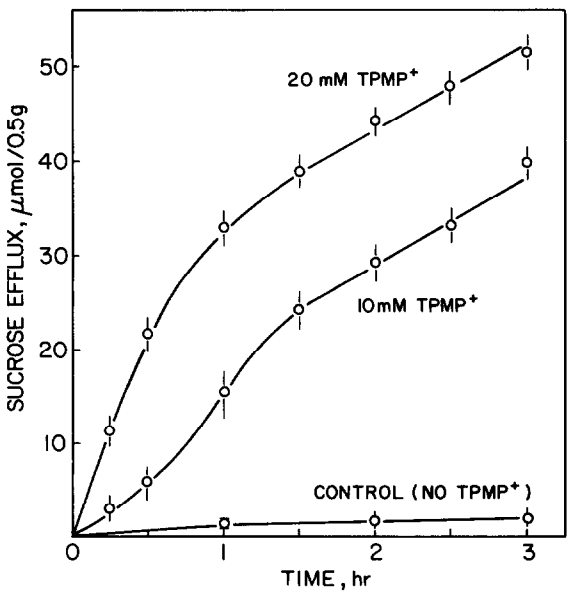

Fig. 3. Sucrose efflux during extended incubation periods in $TPMP^+$. Bathing solution pH was 8. Data are averages from 3 experiments.

Sucrose efflux in the presence of sodium and tetraphenylborate ions

The effect of tetraphenylborate ion (TPB^-) on sucrose efflux appears to be an example of a lipid-soluble ion acting as a porter in a membrane. Na^+ (110 mM) could not serve as a compensating cation for sucrose–proton efflux unless TPB^- (0.5 mM) was present (Fig. 4). When only 10 mM Na^+ was present (the amount added with the buffer), TPB^- had no effect on sucrose efflux. A pH of 8 was required for high rates of sucrose efflux when Na^+ plus TPB^- were used just as it was when $TPMP^+$ was used, although at pH 7, Na^+ plus TPB^- supported a low rate of efflux after a 30-min delay (Fig. 4). The pH effect, therefore, appears to be involved with the sucrose efflux mechanism itself and not with movement of the compensating cation.

Previously, we reported that Na^+, K^+ and Rb^+ could act as compensating cations for sucrose–proton efflux in the absence of TPB^- [3]. At 50 mM, these cations were about as effective as 10 mM $TPMP^+$. However, the slices used in those experiments did not receive the 2-hr fructose and 1-hr mannose incubations, but were placed directly in a buffered (pH 8) mannose solution containing the alkali

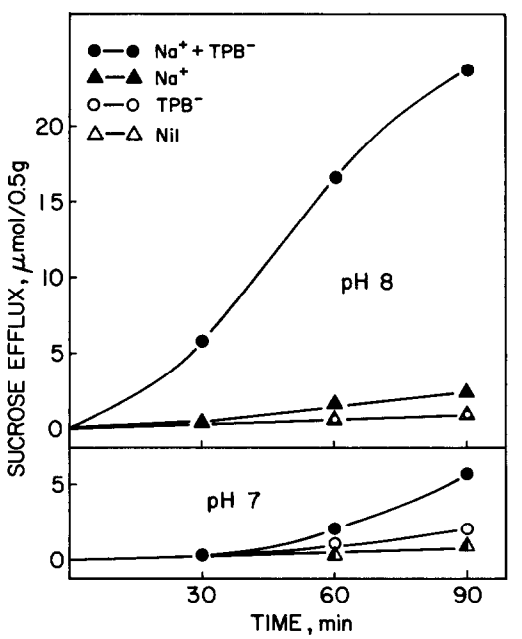

Fig. 4. Effect of Na^+ and tetraphenylborate ions on sucrose efflux. All bathing solutions contained about 10 mM Na^+ which was added with the buffer. NaCl (100 mM) and Na tetraphenylborate (0.5 mM) were added as indicated in the figure. Data are averages from 2 experiments.

cations. Apparently, the scutellum plasmalemma becomes less permeable to alkali cations as a result of the long incubations. It is not known if the loss of permeability reflects a change in the porter systems for alkali cations. However, after long incubation periods, exchange of K^+ for Rb^+ across the plasmalemma goes on at a rapid rate [3].

Generation of a transmembrane electrical potential during sucrose efflux

Because mannose is an energy poison in scutellum cells, mannose treatment should decrease or abolish the electrical potential gradient across the plasmalemma. If sucrose efflux is electrogenic, as the evidence suggests [3], sucrose efflux should generate a membrane potential (negative inside) even in the presence of mannose, and the potential should cause $TPMP^+$ to accumulate in the cell. The extent of $TPMP^+$ accumulation in the tissue from 10 mM $TPMP^+$ in the bathing solution is shown in Table 2. At pH 7, the rate of sucrose efflux was very low, and $TPMP^+$ concentration was the same in both tissue and bathing solution. At pH 8, the rate of sucrose efflux was high, and after 90 min, the tissue $TPMP^+$ concentration was 4 times that of the bathing solution, indicating a transmembrane electrical potential (Nernst potential) of 36 mV, interior negative, and providing direct evidence that sucrose efflux is electrogenic. It is probable that $TPMP^+$ short-circuited the membrane by acting as a porter for anions across it, and therefore, the full capability of sucrose–proton efflux for generating an electrical potential was not realized. It is not clear to what extent the ability of lipid-soluble ions to act as porters limits their usefulness for measuring membrane potential, although $TPMP^+$ has been used for this purpose with bacteria [5], algae [10] and higher plants [4].

Stoichiometry of sucrose–proton cotransport in the direction of efflux

If protons are cotransported with sucrose during sucrose efflux they would react with the buffer in the bathing solution, and their quantity could be determined by titration with alkali. In the experiments of Table 3, proton efflux was determined in this way. By varying the $TPMP^+$ concentration (10 and 20 mM) and the duration of the efflux period (15–90 min), a range of values for sucrose and proton efflux was obtained. In all cases (7 individual analyses from 3 experiments) the proton–sucrose stoichiometry was close to 1.0 (Table 3).

A stoichiometry of 1 has been determined for the galactoside–proton cotransport system of *E. coli* [11] and for the glucose–proton cotransport system of *Chlorella* [12]. However, Komor [4] calculated a proton–sucrose stoichiometry of only 0.3 for sucrose uptake in castor bean cotyledons. Apparently, passive uptake of sucrose (not coupled to proton uptake) in mesophyll cells of the cotyledons caused the low value, although sucrose–proton cotransport took place in the phloem tissue. The values in Table 3 appear to be the most reliable estimates yet made for the stoichiometry of a sugar–proton cotransport system in higher plants; but these values are for the efflux direction, and it is conceivable that there is a different stoichiometry in the influx direction. For instance, the stoichiometry may be pH dependent [9], but this can not be determined by measuring stoichiometry in the efflux direction.

If the stoichiometry is 1, only about one-half of the protons leaving the cell could have been balanced by uptake of $TPMP^+$ ions (Table 2); the rest must have been balanced by anion efflux as was discussed above. Exchange of protons for $TPMP^+$ ions or loss of protons along with anions of a strong acid should increase the

Table 2. Accumulation of $TPMP^+$ in slices*

Bathing soln pH	Incubation period (min)	Sucrose efflux (μmol)	$TPMP^+$ in slices (μmol)	$TPMP^+$ in slices (mM)
7	90	2.5	3.3 ± 1.5	10
8	60	21.1	10.5 ± 2.0	32
8	90	29.4	13.0 ± 2.1	40

* Data are averages from 3 expts.

Table 3. Stoichiometry of sucrose–proton cotransport in the direction of efflux*

Buffer (pH 8)	$TPMP^+$ concn (mM)	Efflux duration (min)	Efflux Sucrose (μmol)	Efflux H^+ (μequiv.)	H^+–sucrose
Bicine	20	15	10.4	8.5	0.82
Bicine	20	60	33.4	33.0	0.99
Bicine	10	90	17.4	19.5	1.12
Epps	20	15	10.6	10.5	0.99
Epps	20	60	33.3	32.4	0.97
Epps	20	75	36.7	34.5	0.94
Epps	10	90	23.6	19.5	0.83

* At the end of the indicated efflux period, sucrose and proton efflux were measured as explained in the Experimental. Data are from 3 expts.

titratable acidity of the tissue, whereas loss of protons along with anions of a weak acid should have no effect on titratable acidity. During a 1-hr sucrose efflux period at pH 8, $TPMP^+$ (20 mM) caused an efflux of 28.6 μmol of sucrose and increased the titratable acidity of the tissue by 9 μequiv (Table 4). Based on a proton transported/sucrose transported stoichiometry of 1, about two-thirds of the proton efflux must have been balanced by the efflux of anions of weak acids. Incubation at pH 7 with 20 mM $TPMP^+$ had essentially no effect on sucrose efflux or titratable acidity (Table 4). These results are consistent with the idea that anion efflux, facilitated by $TPMP^+$, is required to maintain electrical neutrality during sucrose–proton efflux.

EXPERIMENTAL

Preparation of scutellum slices. Maize grains (*Zea mays* L. cv Funks 4949A) were soaked in running tap-water for 24 hr, then placed on moist paper towels and grown in the dark at 24–25° for 72 hr. Scutella were excised and cut transversely with a razor blade into slices of 0.5 mm or less in thickness (3–7 cells in thickness [13]). Slices were washed in H_2O until washings were clear, blotted on filter paper, and weighed into groups of 0.5 g.

Incubation conditions. All incubations were carried out on a Gyrotory water bath (New Brunswick Scientific Co., New Brunswick, NJ) at 30°. Slices were placed in 50-ml beakers with H_2O (10 ml), incubated for 30 min, washed once with H_2O and then the experimental solutions (10 ml) were added. For sucrose efflux expts, slices were incubated in 100 mM fructose for 2 hr; in buffered, 50 mM mannose for 1 hr; and finally, in buffered, 50 mM mannose containing either triphenylmethylphosphonium bromide or sodium tetraphenylborate plus NaCl for 1–3 hr. For sucrose synthesis expts (Table 1) slices were incubated in buffered, 100 mM fructose for 2 hr, and then a portion of the bathing soln was removed for sucrose analysis and the slices were killed in boiling 80% EtOH. The buffers (20 mM) used in the experimental solutions were the Na^+ salts of citrate (pH 3, 4 and 5), Mes (pH 6), Mops (pH 7), and Epps or Bicine (pH 8).

Sugar analysis. Methods for the analysis of sugars in scutellum tissue and bathing solutions have been described [14]. Glucose contents of the bathing solutions were small (negligible above pH 6), and glucose was considered to originate from the action of a bound invertase in the scutellum apoplast [15] on sucrose in the bathing solution. Therefore, for the efflux expts, where the amount of sucrose crossing the plasmalemma is the significant variable, the sum of the glucose and sucrose contents of the bathing soln is reported as sucrose.

Measurement of $TPMP^+$. The $TPMP^+$ content of the bathing solns (Table 2) was determined by the method of Harold and Papineau [16]. Portions of the bathing solns were removed for $TPMP^+$ assay 1.0 min and 60 or 90 min after adding 10 mM $TPMP^+$ to the slices. The decrease in $TPMP^+$ content of the bathing soln was considered to be equal to the $TPMP^+$ content of the slices. $TPMP^+$ concn in the slices was calculated assuming 65% of the fr. wt was cell water in which $TPMP^+$ was uniformly distributed (dry wt of the slices was 30% of fr. wt).

Measurement of proton efflux and proton–sucrose stoichiometry. Groups of slices were incubated in sets of 2 in buffer (pH 8.00) plus 50 mM mannose. In addition, one group in each set received $TPMP^+$. At the end of the incubation, enough KOH (0.01 or 0.05 N) was added to the beakers to bring the pH of the bathing soln back to 8.00, and then portions of the bathing solns were collected for sucrose determination. Within each set, the differences in OH^- titer and the difference in sucrose efflux between the minus and plus $TPMP^+$ bathing solns were considered to be equiv. to proton efflux and sucrose efflux resulting from $TPMP^+$ treatment, and these values were used to calculate the proton–sucrose stoichiometry (Table 3).

Measurement of titratable acidity. At the end of a 1-hr sucrose efflux period (Table 4), slices were washed once with H_2O (10 ml) and killed in boiling H_2O (10 ml). The beakers were placed on a hot plate and boiling was continued for 2 min. After cooling, the hot-water extract plus slices was titrated to pH 3.0 with 0.05 N HCl.

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Table 4. Effect of $TPMP^+$ on titratable acidity of scutellum slices*

Bathing soln pH	$TPMP^+$ (20 mM)	Sucrose efflux (μmol)	Sucrose efflux (difference)	H^+ added to the extract (μequiv.)	H^+ added to the extract (difference)
7	–	1.1		96	
7	+	1.5	+0.4	97	+1
8	–	0.8		96	
8	+	29.4	+28.6	105	+9

* Data are averages of 3 expts. S.d.s were less than 2% (H^+ added) or 12% (sucrose efflux) of the means.