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A MODEL FOR SUCROSE TRANSPORT IN THE MAIZE SCUTELLUM*

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; membrane transport; sucrose transport; proton transport.

Abstract—A model originally developed for transport of neutral substrates in bacterial systems was tested for its suitability for depicting sucrose transport across the plasmalemma of the maize scutellum cell. The model contains a sucrose–proton symporter, a negatively-charged free carrier and a neutral sucrose–proton–carrier complex. Sucrose transport is driven by the sucrose gradient and by a proton electrochemical gradient set up by a proton-translocating ATPase. The results of experiments on sucrose uptake in scutellum slices are in accord with predictions based on the model. Evidence was obtained for an electrogenic proton pump in the plasmalemma, for sucrose–proton symport and for a sucrose transport mechanism driven by both electrical potential and pH gradients. It was found that treatments (dinitrophenol, *N*-ethylmaleimide or HCl) causing a net proton influx into the slices also caused an efflux of sucrose. Interpretations of these results compatible with the model are given.

INTRODUCTION

Mitchell [1] first suggested that β-galactoside transport in *Escherichia coli* may be catalyzed by a proton–galactoside symporter, and West and Mitchell [2, 3] demonstrated the inflow of protons and lactose in a 1:1 stoichiometry into anaerobic, non-metabolizing *E. coli*. According to these workers, the forces driving lactose uptake are the pH difference and electrical potential difference [4] across the membrane. Supposedly, both of these forces are generated by a proton pump (a reversible ATPase [5] or a redox pump of the respiratory chain [6]) in the cell membrane.

A model for a co-transport carrier for neutral substances that incorporates the above ideas is shown in Fig. 1. This model is essentially the same as that developed by Rottenberg [7] for bacterial transport systems. The experiments presented in this paper test the suitability of the model for depicting sucrose transport across the plasmalemma of the maize scutellum cell.

The model contains a carrier (C) that combines with sucrose (S) and protons (H^+) and catalyzes their transport across the membrane. It does not indicate how this is accomplished, whether movement is by translation, rotation or conformational change. The carrier–sucrose–proton complex, being neutral, can move across the membrane in either direction. The uncomplexed, negatively-charged carrier can also move in either direction, but its movement is restrained or facilitated depending on the polarity and magnitude of the membrane potential. The protonated carrier, although neutral, cannot move across the membrane; if it could, it would work against the ATPase and lower or collapse the proton electrochemical gradient. The proton pump is depicted as a reversible ATPase. This seems a more likely assumption than the presence of a redox pump in the plasma membrane of a eucaryotic cell. The ATPase diagram shows two protein components and the 'proton well', properties that stress the 'sidedness' of the enzyme in the membrane [5]. The ATPase diagram is based on work with mitochondrial, chloroplastic and bacterial ATPases, and it may not accurately depict the plasmalemma enzyme.

The following predictions from the model were tested using slices of the maize scutellum: (1) sucrose uptake is pH dependent, (2) the presence of a proton pump and (3) sucrose fluxes are accompanied by proton fluxes.

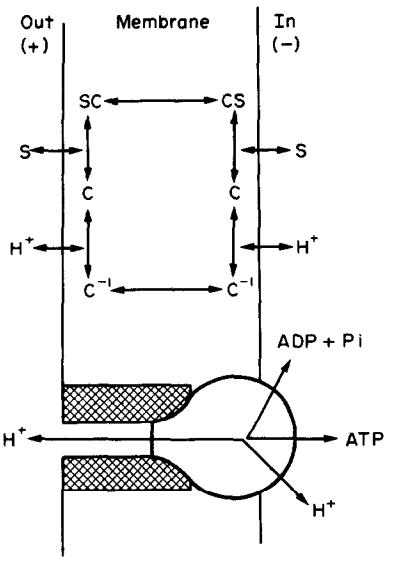

Fig. 1. A model for sucrose transport.

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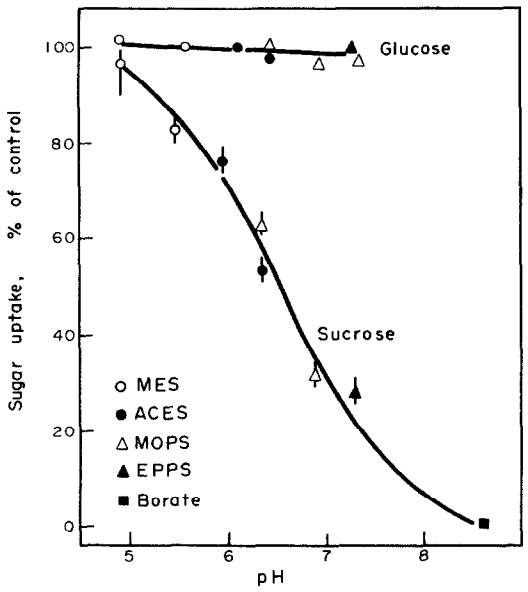

Fig. 2. Effect of pH on sugar uptake (for key to buffer abbreviations see Experimental).

RESULTS AND DISCUSSION

Effect of pH on sucrose uptake

The results of Fig. 2, which are in agreement with the model, show that the rate of sucrose uptake declined as the pH was increased above 5. Above pH 8.5 the rate was essentially zero. In contrast to the results with sucrose, pH had no effect on glucose uptake in the range 5 to 7.5 (Fig. 2). This result is in accord with previous work that indicates the scutellum plasmalemma is a negligible barrier to free diffusion of hexoses [8, 9].

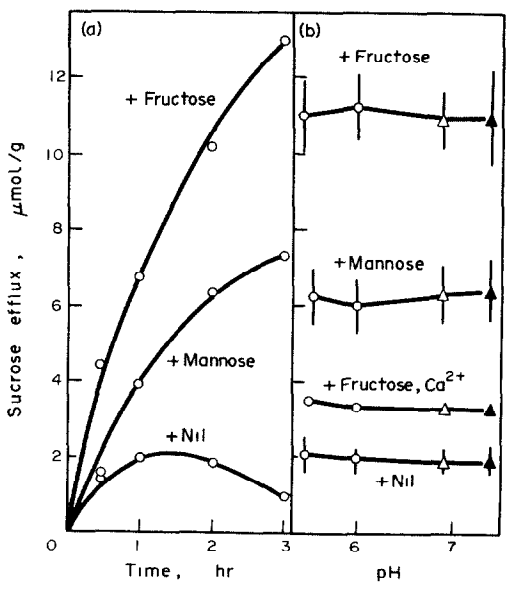

Fig. 3. Effect of pH on sucrose efflux. In (a) portions of the bathing solutions were removed for analysis. (b) shows the amount of sucrose in the bathing solutions after 2 hr.

In the experiments of Fig. 2, sucrose uptake is the difference between sucrose influx and efflux whereas, to test the model of Fig. 1, the rates of sucrose influx should be determined. If the rate of efflux was low in comparison to influx and was unaffected by bathing solution pH, then the curve of Fig. 2 would closely approximate the true influx rate vs pH curve. It was not possible to measure sucrose efflux during sucrose uptake. Instead, slices were placed in buffer, buffered fructose ($\pm CaCl_2$) or buffered mannose, and the amount of sucrose efflux was followed during a 3 hr period (Fig. 3). Each solution supported a different rate of efflux, and the rates were essentially independent of external pH in the range 5.4 to 7.4 (Fig. 3). Slices placed in buffer alone exhibited a very low rate of sucrose efflux even though they contained 60 to 70 μmol sucrose per g. Apparently, most of this sucrose was sequested in the vacuoles, and only a small amount was in the cytoplasm whence it was available for efflux. This idea is supported by results obtained when mannose and fructose were added to the bathing solutions. Both of these hexoses increased the rate of efflux. Mannose causes a slow release of vacuolar sucrose into the cytoplasm [10], and in the presence of fructose the slices rapidly synthesized sucrose (30 to 35 μmol/g/hr). Sucrose efflux was strongly inhibited when $CaCl_2$ was added to the fructose (Fig. 3) or mannose (data not shown) solutions.

The above results indicate that efflux is controlled by the level of cytoplasmic sucrose, not by the level of total tissue sucrose. If it is assumed that the sucrose efflux rate is proportional to the cytoplasmic sucrose concentration, and that this, in turn, is proportional to the rate of sucrose influx, the following relationships will hold. (1) $E = k_0C$; (2) $C = k_1I + C_0$; (3) $E_0 = k_0C_0$; (4) $U = I\text{-}E$; and, therefore, (5) $U = I(1\text{-}k)\text{-}E_0$. Where E is the efflux rate, I is the influx rate, E_0 is efflux rate when $I = 0$, U is the uptake rate, C is the cytoplasmic sucrose concentration, C_0 is the cytoplasmic sucrose concentration when $I = 0$, the k's are constants and $k = k_0k_1$. E_0 is a constant over the pH range 5.4 to 7.4 (Fig. 3, + nil). If k is also a constant over this pH range, the influx vs pH curve would be very similar to the uptake curve of Fig. 2, but it would not reach zero. However, since the rates of sucrose metabolism and transport will also be influenced by the influx rate, k_1 (equation 2) is probably a constant only at relatively low influx rates. At sucrose influx rates that saturate the metabolic and transport processes, k_1 should increase. If this occurred, the influx vs pH curve would have the same general shape as the uptake curve of Fig, 2, but it would decline more rapidly as the pH increased and would not reach zero. It is doubtful that the maximum uptake rate in the experiments of Fig. 2 (15 to 17 μmol sucrose/g/hr) exceeded the capacities of the scutellum cells for metabolizing and transporting sucrose. In 0.1 M fructose, scutellum slices utilize about 20 μmol fructose/g/hr for respiration and fermentation and about 60 μmol/g/hr for sucrose synthesis [11, 12]. Only small amounts of this sucrose are released from the slices (e.g. Fig. 3), and most of it is transported into storage [13]. It is concluded from the above analysis that the sucrose uptake curve of Fig. 2 closely approximates the true sucrose influx curve.

Rottenberg [7] derived an equation (based on the model of Fig. 1) that states that the rate of net transport is proportional to the sum of the chemical potential

gradients of protons and neutral substrate. In the experiment of Fig. 2, only the chemical potential of protons was varied. Assuming external pH affects only the degree of protonation of the carrier at the external face of the plasmalemma (i.e. it has no effect on sucrose activity, binding of sucrose to the carrier, cytoplasmic pH, or membrane electrical potential), the curve of Fig. 2 indicates that the proton-binding group of the carrier has a pK of about 6.6. This estimate of pK probably is too high because under the influence of a membrane potential, negative inside, the proton activity at the external surface of the plasmalemma would be higher than that in the bulk solution where the pH is measured. Furthermore, it has been shown with algae that as the external pH is increased both the membrane potential and the cytoplasmic pH increase [14]. If these changes also occur in the scutellum cell, the amount by which the curve of Fig. 2 overestimates the pK would be increased. A pK of 6.6 or below indicates that the proton-binding group of the carrier might be a carboxyl, imidazole or phosphate group. Protonation of a carboxyl or phosphate group would neutralize a negative charge (as specified in the model of Fig. 1) whereas protonation of an imidazole group would introduce a positive charge. Kaback *et al.* [15] presented evidence that the lactose carrier in *E. coli* membrane vesicles is negatively charged.

The proton pump

The existence of a proton pump in the plasmalemma was indicated by the ability of scutellum slices to acidify the bathing solution. Slices were placed in water, sucrose or $CaCl_2$, and the bathing solutions were maintained at pH 6 by adding NaOH at 10 min intervals (Fig. 4). The rate of NaOH addition gives the apparent rate of proton efflux, although OH^- influx would give the same result and could not be distinguished from proton efflux. The rate of proton efflux into sucrose was the same as that into water, but $CaCl_2$ caused a 3.5-fold increase in rate. KCl, K_2SO_4 and NaCl also greatly increased the apparent proton efflux rate (results not shown). The stimulatory effect of these cations on proton efflux is usually taken to mean that the proton pump can produce a transmembrane electrical potential difference, inside negative [16]. The movement of Ca^{2+}, K^+ and Na^+ into the cell would reduce the electrochemical gradient of protons against which the pump is working, thereby increasing the pumping rate. Similarly, according to the model (Fig. 1), cotransport of protons during sucrose uptake works against the proton pump, but it also increases the pumping rate by decreasing the proton electrochemical gradient. Apparently, these two opposing factors balance at pH 6 since 20 mM sucrose had no effect on proton efflux at this pH (Fig. 4).

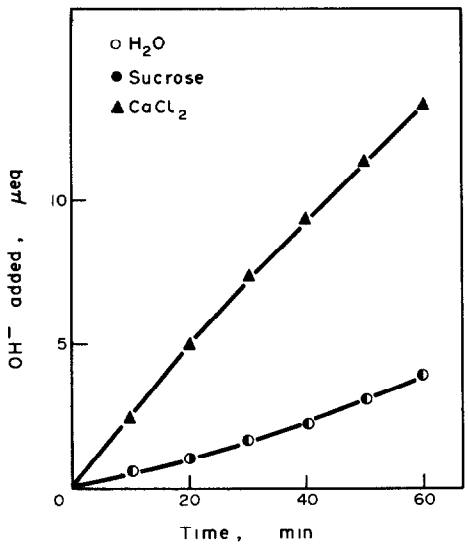

Fig. 4. Apparent proton efflux scutellum slices.

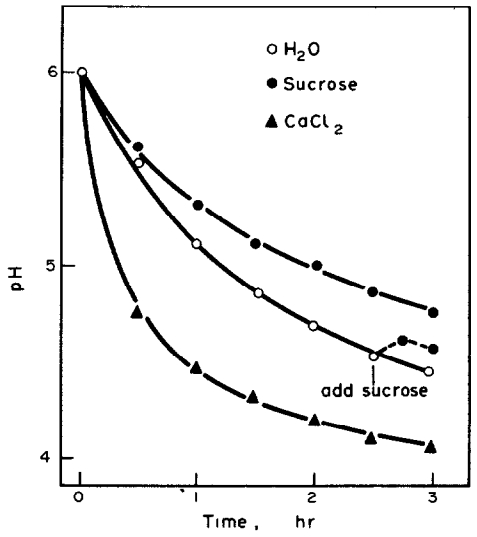

Fig. 5. Change of bathing solution pH with time after adding scutellum slices.

Since the apparent rate of proton efflux decreases below pH 6 [17] whereas the rate of sucrose uptake increased (Fig. 2), the effect of sucrose on the rate of decline of bathing solution pH was determined (Fig. 5). In agreement with the model (Fig. 1), addition of sucrose to the bathing solution decreased the rate of pH decline. Addition of sucrose (to a final concentration of 20 mM) 2.5 hr after slices to water caused a small, but transitory, increase in pH (Fig. 5). A large· sustained increase in pH would not be expected since it would require either simultaneous influx of an anion or efflux of a cation. These fluxes would probably not occur unless the membrane potential was abolished or its polarity reversed. If this occurred, sucrose uptake should be a self-inhibitory process, and the rate of sucrose uptake should decrease with time. It has been shown that this does not happen in the maize scutellum [12]. On the contrary, when the external sucrose concentration was held constant, the rate of sucrose uptake increased with time [12].

It is assumed in the experiments of Figs 4 and 5 that a steady state is rapidly reached between the concentrations of HCO_3^- and CO_2 in the slices and bathing solution and the CO_2 of the air. This assumption is supported by the fact that sucrose increases CO_2 production [12] but not acidification of the bathing solution (Figs 4 and 5) and

Table 1. Calcium and sucrose uptake*

Buffer	pH range	Ca^{2+}	Sucrose uptake μmol/g/hr	% Control (minus Ca^{2+})
Nil	5.8–4.9	–	14.9 ± 1.2	
Nil	5.8–4.4	+	16.8 ± 0.8	113
Mes	5.0–5.0	–	15.8 ± 1.6	
Mes	5.0–4.8	+	14.6 ± 0.7	92
Mes	6.1–6.0	–	11.7 ± 0.5	
Mes	6.1–6.0	+	7.0 ± 0.8	60

* Sucrose uptake was determined as described in the Experimental. The values for sucrose uptake are averages from the results of three experiments followed by the standard deviation. The concn of $CaCl_2$ was 20 mM and of buffer was 25 mM. The 'pH range' column gives the initial and final bathing soln pH.

that acidification continues in all cases even below pH 5 (Fig. 5).

Ca^{2+} greatly increased the rate of pH decline (Fig. 5), in accord with its effect on proton efflux at pH 6 (Fig. 4). The results of Figs 4 and 5 are interpreted to mean that Ca^{2+} decreases the membrane potential. If this interpretation is correct, Ca^{2+} should inhibit sucrose uptake. The results of Table 1 show that Ca^{2+} inhibited sucrose uptake by 40% at pH 6 but only by 8% at pH 5. These results are in agreement with the above interpretation of the action of Ca^{2+} since, as the pH is lowered, membrane potential should make a smaller contribution to the proton electrochemical gradient. In the absence of buffer, Ca^{2+} caused a small, but reproducible, increase in the rate of sucrose uptake (Table 1). The increase probably was a consequence of both the more rapid decline of pH in the presence of Ca^{2+} (Table 1, Fig. 5) and the inhibition of sucrose efflux by Ca^{2+} (Fig. 3). This result suggests that only low rates of sucrose efflux were occurring during sucrose uptake.

The results of this section are in accord with the predictions of the model (Fig. 1). They provide evidence for an electrogenic proton pump in the plasmalemma (Figs 4 and 5), for a sucrose-proton symport (Fig. 5) and for a sucrose transport mechanism driven by both electrical potential and pH gradients (Table 1).

Sucrose efflux

Previously, it was shown that dinitrophenol (DNP) induces a proton influx into maize scutellum slices [17] and that proton influx is accompanied by sucrose efflux [13]. Treatment of slices in *N*-ethylmaleimide (NEM) and in HCl at pH 2 also resulted in proton influx and sucrose efflux (Figs 6 and 7). The time courses of the sucrose and proton fluxes induced by DNP and NEM are shown in Fig. 6. DNP was tested at pH 5, 4 and 3.5, and both proton influx and sucrose efflux increased as the pH was lowered. For the NEM experiment, slices were pretreated with NEM, washed in water and placed in water maintained at pH 4 with HCl. The rates of proton influx and sucrose efflux also increased as the pH was lowered with NEM-treated slices (data not shown). The effects of HCl on proton and sucrose fluxes

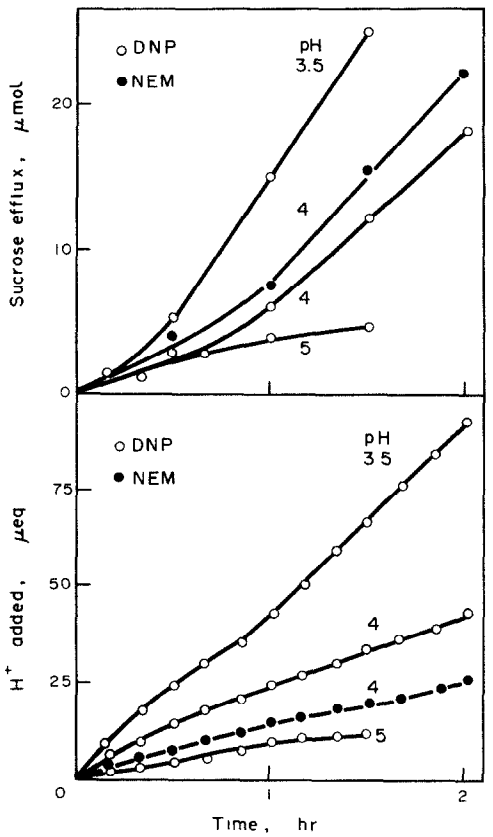

Fig. 6. Induction of proton influx and sucrose efflux by DNP and NEM.

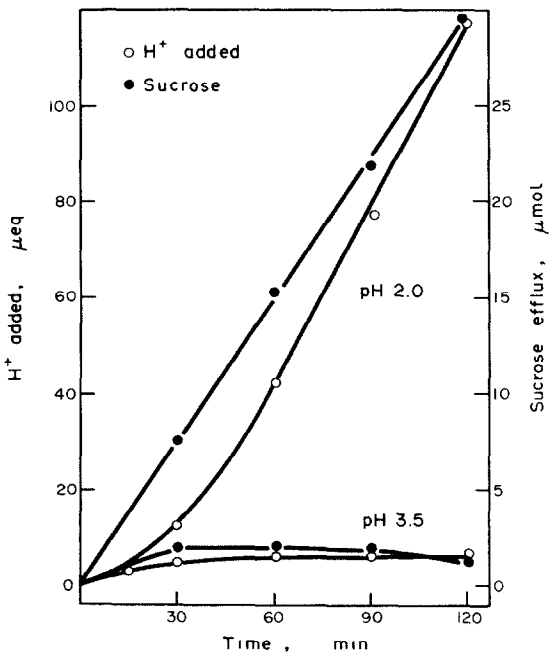

Fig. 7. Proton influx and sucrose efflux in the presence of HCl

Table 2. Effect of HCl on sucrose efflux and production*

Slices	pH range	Sucrose, μmol/g				
		Effluxed	+	Tissue change	=	Total
Fresh	5.7–4.8	30 ± 2.1	+	47 ± 3.5	=	77
Fresh	2.0–2.2	55 ± 4.7	+	1.8 ± 4.6	=	57
Water-treated	5.1–4.4	7 ± 1.3	+	64 ± 1.7	=	71
HCl-treated	3.6–3.5	11 ± 2.0	+	62 ± 2.6	=	73

* Fresh slices (0.5 g) were incubated in 10 ml of 0.1 M fructose or 0.1 M fructose plus HCl (pH 2) for 24 hr. At the end of the incubation, portions of the bathing solns were removed for the determination of sucrose efflux, and the slices were killed in boiling 80% EtOH to determine tissue sucrose. The amount of sucrose in slices killed at the beginning of the incubation was used to determine the change in tissue sucrose. Water and HCl treatments consisted of incubation of slices in water or HCl (pH 2) for 2 hr. At the end of the treatments, the slices were washed twice with water (10 ml), and incubated in 0.1 M fructose for 2 hr. Sucrose efflux and tissue sucrose were determined as above with the fresh slices. Slices killed at the end of the water and HCl treatments were used to determine changes in tissue sucrose. The sucrose values are averages of four experiments followed by the standard deviation.

are shown in Fig. 7. At pH 2, high, sustained rates of sucrose efflux and proton influx were obtained over a 2 hr period. At pH 3.5, both fluxes occurred at low rates for 30 min and then stopped. With all treatments (DNP, NEM and HCl) proton influx was greater than sucrose efflux, both fluxes increased as the pH was lowered; and except for HCl at pH 3.5, the two fluxes had different time courses.

Sucrose efflux at pH 2 could be the result of injury. To test for injury, slices were incubated in fructose during and after HCl treatment, and sucrose production was measured (Table 2). Sucrose production in slices incubated in HCl (pH 2) plus fructose for 2 hr was 74% as great as that in control slices incubated in fructose alone. Slices pretreated in HCl (pH 2) for 2 hr before being placed in fructose produced sucrose at essentially the same rate as control slices pretreated in water. The most striking difference between fresh and pretreated slices was in the rates of sucrose efflux that took place during sucrose synthesis (Table 2). The amount of sucrose efflux from slices pretreated in either water or HCl was only 10 to 15% of the sucrose produced whereas sucrose efflux from fresh slices was 39% (fructose) and 97% (fructose plus HCl) of that produced. Efflux of sucrose from control slices (those not treated in HCl) occurred without an accompanying proton influx. From the above results, it appears unlikely that sucrose efflux was caused by injury.

Proton efflux, whether caused by sucrose efflux or by operation of the proton pump (Fig. 1), would require an essentially equal efflux of anions or influx of cations. The near equality of the fluxes is stipulated because membrane potentials of the magnitude found in cells are not large enough to account for marked departures from electrical neutrality. For instance, according to the calculations of Nobel [18] a potential of 200 mV across the plasmalemma (a value on the high side of the range of potentials measured in higher plants [19]) would require less than 1 neq of excess charge per g fresh wt of tissue.

From the above discussion and assuming the correctness of the model in Fig. 1, the sucrose efflux experiments (Figs 6 and 7, Table 2) are interpreted as follows: (1) Sucrose efflux occurs as sucrose-proton symport. (2) The forces driving sucrose efflux are the sucrose and pH gradients and the membrane potential. (3) Proton influx causes sucrose efflux by abolishing the membrane potential or reversing its polarity (inside positive) and by providing protons for sucrose-proton symport. In these experiments, sucrose efflux occurred against the pH gradient. During proton influx, therefore, the forces driving sucrose efflux were the sucrose gradient and the membrane potential. (4) In the absence of a proton influx (control slices, Table 2), sucrose efflux was driven by the sucrose gradient alone, and efflux occurred against the pH gradient and the membrane potential. In these experiments, the sucrose gradient was determined primarily by the cytoplasmic sucrose concentration. The amount of sucrose in the cytoplasm is determined by the initial concentration, and by rates of sucrose synthesis, sucrose transport into storage vacuoles and sucrose efflux across the plasmalemma. Fresh slices have a higher initial sucrose concentration in the cytoplasm than do those pretreated in water [10], and this probably accounts for the higher rate of sucrose efflux when fresh slices were placed in fructose (Table 2).

Proton influx requires comment. In a previous paper [17], the idea that DNP is the carrier of protons across the membrane was depreciated. Instead, it was suggested that DNP-induced proton influx resulted from reversal of the proton pump. The results with NEM support this suggestion (Fig. 6). NEM induced an apparent proton influx, but NEM is not a weak acid, although on hydrolysis it yields the weak acid, *N*-ethylmaleamate. However, since a pretreatment procedure was used in the experiment of Fig. 6 and since hydrolysis of NEM is negligible at the low pH (4 to 5) of the NEM treatment solution [20], it is unlikely that NEM-induced proton influx represents an NEM or *N*-ethylmaleamate shuttle across the plasmalemma. NEM, being a powerful SH-reagent, inhibits many enzymes upon which DNP has no effect. However, both NEM and DNP inhibit oxidative phosphorylation and stimulate Mg^{2+}-ATPase [21–23]. Thus, NEM and DNP might cause an increase in the ADP level inside the plasmalemma which, coupled with a high proton activity outside (pH 5 or below, Fig. 6), could result in reversal of the ATPase reaction. Acid-induced proton influx (HCl, pH 2; Fig. 7) probably resulted from passive diffusion of protons across the membrane.

In conclusion, the results of this paper suggest that the mechanism for sucrose transport across the maize scutellum plasmalemma can be described by the model shown in Fig. 1. However, in previous papers [9, 10] it was concluded that the sucrose transport system of the maize scutellum does not release free sucrose into the cytoplasm. This conclusion was based primarily on the observation that scutellum slices bathed in M sorbitol were unable to utilize or transport cytoplasmic and vacuolar sucrose but could transport and utilize exogenous sucrose. It appears that the model requires some modification, perhaps the addition at the inner face of the plasmalemma of a reaction for the splitting of sucrose. This presently is under investigation.

Tanner [24] presented a model for hexose transport in *Chlorella vulgaris* that contains a sugar-proton symporter, although it differs from the model of Fig. 1.

The *Chlorella* system has a stoichiometry of 1 proton taken up per sugar molecule [25], and the chemical potential difference of protons across the membrane is sufficient to account for the observed sugar accumulation [26]. Evidence has been obtained for hexose–proton symport during active uptake in wheat roots [27], and for sucrose–proton symport during phloem loading in in leaf discs of sugarbeet [28].

EXPERIMENTAL

Maize grains (*Zea mays* L., cv G4949, Louisiana Seed Co., Alexandria, Louisiana, USA or cv McNair 508, McNair Seed Co., Laurinburg, North Carolina, USA) were soaked in running H_2O for 24 hr and then placed on moist paper towels in the dark at 24–25° for 72 hr. Scutella were excised and cut transversely into slices 0.5 mm or less in thickness. Slices were washed until the washings were clear, blotted on filter paper and weighted in groups of 0.5 or 1 g. All incubations were carried out at 30°. An Orion, model 701, digital pH meter was used for pH measurements. Methods for the analysis of sugars in scutellum tissue and bathing solns have been described [29].

Effect of pH on sugar uptake. Slices (0.5 g) were incubated in H_2O (10 ml) for 30 min and washed once. 10 ml of 5 mM sugar (in 25 mM buffer) was added, and the slices incubated for 2 hr. Portions of the bathing solns, taken at the beginning and end of the sugar incubation, were used to determine sugar uptake. The uptake rates in buffer are given in Fig. 2 as % rate of uptake in control slices incubated in sugar alone. Average control rates in μmol/g/hr were 15.4 for sucrose and 22.1 for glucose. Each point on the sucrose curve represents an average of the results of three or four experiments, and the line perpendicular to each point gives the range of values obtained. The values for glucose uptake are averages from the results of two experiments. The pH of each soln was taken at the beginning and end of the incubation, and the average of these two values was used to plot the curve. The pH decline of the buffered solns was less than 0.3 unit except for borate which dropped from 9.1 to 8.2. The pH in the control flask was 5.8 at the beginning and 4.8 at the end of the incubation. The buffers were Mes (2[*N*-morpholino] ethane sulfonic acid), Aces (*N*-[2-acetamido]-2-aminoethane sulfonic acid), Mops (morpholinopropane sulfonic acid), and Epps (4[2-hydroxyethyl]-1-piperazine propane sulfonic acid).

Effect of pH on sucrose efflux. Slices (0.5 g) were incubated in H_2O (10 ml) for 30 min and washed once. 10 ml of 25 mM buffer, buffer plus fructose (0.1 M) ± 20 mM $CaCl_2$, or buffer plus mannose (0.05 m) was added. In Fig. 3a, the buffer was Mes, pH 6.1. Portions of the bathing solns were removed for sucrose analysis at the times shown. In Fig. 3b, amounts of sucrose in the bathing solns after 2 hr (after 1 hr in + nil) are shown. Averages of 3 experiments are plotted and the standard deviations are indicated (the values for fructose plus $CaCl_2$ are averages of 2 experiments)

Apparent proton efflux from scutellum slices. Slices (1 g) were incubated in 17 ml H_2O, sucrose (20 mM) or $CaCl_2$ (20 mM). The pH was maintained at 6 by addition of 10 mM NaOH at 10 min intervals. Results of a single experiment are shown in Fig. 4. The experiment was run × 3 and the rates of NaOH addition deviated 8% or less from those shown.

Change of bathing soln pH with time after adding scutellum slices. Slices (1 g) were incubated in 17 ml H_2O, sucrose (20 mM) or $CaCl_2$ (20 mM). The solns were adjusted to pH 6 (with NaOH) 10 min after adding the slices (time zero on graph). Sucrose (1 M, 0.34 ml) to a final concn of 20 mM was added to the H_2O in one flask 2.5 hr after adding slices. Results of a single experiment are shown in Fig. 5. In three experiments, (two experiments, $+CaCl_2$) similar results were obtained, and the final pHs were within 0.15 units of those shown.

Induction of proton influx and sucrose efflux by DNP and NEM. Slices (1 g) were incubated in H_2O or 10 mM NEM for 30 min and then washed twice. The pH of the NEM soln, initially about 4, rose to 5 during the 30 min treatment period. DNP (0.5 mM, 17 ml) was added to H_2O-treated slices and H_2O (17 ml) was added to the NEM-treated slices. Solns were maintained at the pHs indicated by the addition of 20 mM HCl. Portions of the bathing solns were removed for sucrose analysis at the times shown. Efflux of sucrose in the presence of DNP or NEM was accompanied by the appearance in the bathing soln of glucose and fructose in nearly equal amounts. Exogenous sucrose is hydrolyzed by these treatments [9, published observations]; and, therefore it is assumed that sucrose efflux preceded sucrose hydrolysis. In the experiment, sugar efflux (glucose + sucrose) is reported as sucrose efflux. At the end of 2 hr, the bathing soln of the NEM-treated slices contained 9 μmol glucose and 13.5 μmol sucrose. The DNP bathing soln (pH 4) contained 9.3 μmol glucose and 8.5 μmol sucrose. Results of a single experiment for each treatment are shown in Fig. 6. The experiments were repeated 2 to 4 times with similar results.

Proton influx and sucrose efflux in the presence of HCl. Slices (1 g) were incubated in H_2O for 30 min and then placed in 17 ml of HCl at pH 2 or 3.5. Bathing soln pH was maintained by the addition of 100 mM HCl (pH 2) or 20 mM HCl (pH 3.5). Portions of the bathing solns were removed for sucrose analysis at the times shown. HCl (pH 2) caused a slow hydrolysis of sucrose in the absence of scutellum slices. Therefore, in this experiment, sugar in the bathing soln (glucose plus sucrose) is reported as sucrose efflux. At the end of 2 hr, the bathing soln contained 4.4 μmol glucose and 25.2 μmol sucrose. Results of a single experiment are shown in Fig. 7. The experiments were repeated × 3 (pH 2) and × 2 (pH 3.5) with similar results.

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