Plant Physiol. (1980) 66, 884–889
0032-0889/80/66/0884/06/$00.50/0

Alkali Cation/Sucrose Co-transport in the Root Sink of Sugar Beet[1]

Received for publication September 4, 1979 and in revised form June 10, 1980

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ABSTRACT

The mechanism of sucrose transport into the vacuole of root parenchyma cells of sugar beet was investigated using discs of intact tissue. Active sucrose uptake was evident only at the tonoplast. Sucrose caused a transient 8.3 millivolts depolarization of the membrane potential, suggesting an ion co-transport mechanism. Sucrose also stimulated net proton efflux. Active (net) uptake of sucrose was strongly affected by factors that influence the alkali cation and proton gradients across biological membranes. Alkali cations (Na^+ and K^+) at 95 millimolar activity stimulated active uptake of sucrose 2.1- to 4-fold, whereas membrane-permeating anions inhibited active sucrose uptake. The pH optima for uptake was between 6.5 and 7.0, pH values slightly higher than those of the vacuole. The ionophores valinomycin, gramicidin D, and carbonyl cyanide *m*-chlorophenylhydrazone at 10 micromolar concentrations strongly inhibited active sucrose uptake. These data are consistent with the hypothesis that an alkali cation influx/proton efflux reaction is coupled to the active uptake of sucrose into the vacuole of parenchyma cells in the root sink of sugar beets.

The transport and accumulation of sugars in sink tissues of economically important crops is poorly understood. In sugar beet, sucrose is translocated and stored in the vacuole of parenchyma cells in the root sink without hydrolysis (4, 19). However, the mechanism of phloem unloading and the subsequent uptake and storage of sucrose is not understood (19). The important role of the taproot sink in controlling photosynthate partitioning was evident in grafting and photosynthate (sugar) supply studies (18). These studies showed that the root sink of sugar beet, independent of photosynthate supply, controls partitioning between sucrose and non-sucrose organic dry matter. The elucidation of cellular events during sucrose accumulation in the sugar beet root would provide valuable information about photosynthate transport and partitioning in sink tissues and, possibly, about source-sink communication.

Proton co-transport of sugars has been recently substantiated in a number of higher plant tissues. The energy for this transport of sugars may be provided by a gradient of electrochemical potential for protons across a membrane that could be generated and maintained by a vectorial, membrane-bound ATPase (see refs. 1 and 14, and references therein). Evidence indicating a proton/sugar co-transport mechanism driven by a proton gradient in higher plants include: (*a*) proton influxes and alkalinization of the medium during sugar uptake; (*b*) transient changes (depolarizations) in the electrical potential of membranes in response to sugar supply; (*c*) inhibition of sugar uptake by factors that reduce the proton gradient across membranes, such as alkali cations, metabolic inhibitors, and ATPase inhibitors; and (*d*) stimulation of sugar uptake by factors that increase the proton gradient across membranes such as low pH and ATP supply.

Komor (10) and Hutchings (8, 9) have extensively characterized a proton/sucrose co-transport mechanism in *Ricinus* cotyledons, and Giaquinta (3, 5) has prevented strong evidence for the coupling of phloem loading of sucrose with proton transport in source leaves of sugar beet. Sugar uptake has also been correlated with proton movements in *Samanea* pulvini (15), tomato internode (17), and phloem tissue of *Ricinus* petioles (12). However, the ionic gradients existing in source and sink tissues may be quite different. For example, phloem loading of sucrose is from the apoplast which has a low K concentration but a lower pH than the sieve element (7, 21), whereas, in sugar beet sink tissue, the opposite gradient exists between the apoplast and the vacuole of the storage parenchyma cells (unpublished data). In addition, active phloem laoding occurs across the plasmalemma, but active loading in the root parenchyma cell occurs at the tonoplast. Because of these differences between source and sink tissue, the mechanism of sucrose transport in tissue discs from the taproot sink of sugar beet was investigated. The results indicate the existence of an alkali cation/sucrose co-transport mechanism in the root sink of sugar beet. Very recently, van Bel and van Erven (17) proposed a model for proton and K co-transport with sucrose.

MATERIALS AND METHODS

Preparation of Plants. Seeds of *Beta vulgaris* L. (sugar beet) cultivars AH11 and D2 were obtained from The Amalgamated Sugar Co. (Nampa, Idaho) and from the Great Western Sugar Co. (Longmont, Colo.), respectively. Cultivar D2 plants were grown in the greenhouse under the following conditions: 16-h photoperiod (daylight h were extended by use of incandescent bulbs and Gro-Lux fluorescent tubes); about a 25/18 C day/night temperature cycle; 25-liter pots filled with Vermiculite. Cultivar AH11 plants were grown in growth chambers under the following conditions: 16-h day from incandescient bulbs and fluorescent

[1] Cooperative investigations of Agricultural Research (United States Department of Agriculture, Science and Education Administration), the Beet Sugar Development Foundation, and the Utah State Agriculture Experiment Station. Approved as Journal Paper No. 2466. This work was supported by the Science and Education Administration of the United States Department of Agriculture under Grant 5901-0410-8-0187-0 from the Competitive Research Grants Office.

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tubes, Sylvania F96T12-CW-VHO-LT[4] (450 μE m^{-2} s^{-1}); 28/18 C day/night temperature cycle; 25-liter pots filled with a 1:1:1 mixture of locally obtained topsoil, peatmoss, and sand. All plants were fertilized once a week with a complete nutrient solution. Individual plants between 3 and 10 months old were harvested on the days that experiments were conducted. The two cultivars showed no significant differences in the uptake of sucrose. However, younger, more actively growing plants had higher sucrose uptake rates than older, less actively growing plants.

Discs of root tissue were prepared by cutting slices 1 mm thick with a hand microtome. For sucrose uptake and proton efflux studies, discs 5 mm in diameter were cut from these slices with a sharp cork borer. Unless stated otherwise, the discs were washed three times for 1 min each in a large excess of deionized H_2O. The discs then were incubated for 2 to 3 h in aerated, deionized H_2O (15 discs/ml H_2O). During this period, sucrose was released from the discs until the concentration of the ambient solution stabilized at 25 to 33 mM (20).

For electrophysiological studies, the 1-mm slices of sugar beet root were equilibrated for 3 h in a solution of 10 mM KIDA[5] and 1 mM Mops adjusted to pH 7.0 with KOH. About 0.35 g tissue/ml salt solution was used. After the incubation, the tissue slices were trimmed with a razor blade to fit into a rectangular 20- × 7- × 7-mm chamber.

Sucrose Uptake Measurements. The equilibrated discs were divided into sets of 30 discs each. Unless otherwise stated, the sets, in replicates of three, were preincubated for 15 min in a large excess of unlabeled base sucrose solution (40 mM sucrose, 1 mM Mops adjusted to pH 6.5 with KOH) with or without 10 μM CCCP and with or without various treatments being tested for their effect on sucrose uptake.

After the 15-min preincubation, each set of discs was blotted gently with a Kimwipe and transferred to a vial containing the preincubation media plus [^{14}C]sucrose. The specific radioactivity of the sucrose solutions was about 30,000 dpm/μmol sucrose, provided through appropriate dilution of uniformly labeled [^{14}C]sucrose (1.9 mCi/mg sucrose). Sets of discs were incubated at room temperature in the labeled sucrose solutions for 4 to 8 h, depending on the experiment, while humidified air was slowly bubbled through each vial to prevent anaerobiosis. The specific radioactivity of the labeled sucrose solutions did not change significantly during the incubation period. The rate of [^{14}C]sucrose uptake was linear for the first 6 h and then declined somewhat over the next 2 h uptake. However, active uptake into the vacuole did not begin until the cytoplasm had equilibrated with the external media. Linear active uptake showed a lag time of approximately 90 min.

After the incubation, each set of discs was again washed three times for 1 min in a large excess of deionized H_2O to remove labeled sucrose from the cut cells on the surface and about 90% of the sucrose in the free space of the cell wall. Each set of discs was placed in a liquid scintillation vial containing 10 ml 80% ethanol and the preparation was heated to boiling. The vial was capped and allowed to stand overnight at room temperature. Ethanol was removed by boiling and the residue was adjusted to 4 ml with deionized H_2O. Radioactivity was determined by liquid scintillation spectroscopy and efficiency was determined by the channels ratio method. The initial fresh weight of undamaged tissue from 30 unequilibrated discs of sugar beet was about 0.75 g. This value was used to convert sucrose uptake rates onto a fresh weight basis.

Sucrose uptake from base sucrose solutions containing 10 μM CCCP was considered as passive sucrose uptake. CCCP at this concentration inhibited respiration more than 98% and led to no detectable net sucrose uptake; passive sucrose uptake probably involves [^{14}C]sucrose uptake in exchange for unlabeled sucrose. Recent results in this laboratory indicated that there is predominately passive movement of sucrose across the plasmalemma at the 40 mM sucrose concentrations used in these uptake experiments (20). Therefore, metabolically dependent or active sucrose uptake at the tonoplast could be calculated by substracting passive sucrose uptake (uptake in the presence of CCCP) from sucrose uptake in the absence of CCCP.

The effect of the alkali cations K^+ and Na^+ was tested in conjunction with the following anions: IDA^-, Cl^-, NO_3^-, SO_4^{2-}, and $HPO_4^{2-}/H_2PO_4^-$. Anionic effects were tested using HCl, HNO_3, H_2SO_4, H_3PO_4, and IDA^- neutralized to pH 7.0 with BTP.

For the study of proton effects on sucrose uptake, the pH of the base sucrose solution was modified. The base sucrose solutions were buffered at pH 5.0, 5.5, 6.0, 6.5, and 7.0 using 50 mM Mops at pH 8.0 using 50 mM Tricine. Since variable amounts of KOH were required for pH adjustment of the solutions, KIDA was added to the solutions so that the K^+ ion concentration in all the solutions was 20 mM.

Proton Efflux Measurements. Measurements of proton efflux were made with a Sargent-Welch pH-stat using 12.5 mM BTP as the titrant. Discs were washed for 30 min in running tapwater and then equilibrated for 1 h in 1 mM $CaSO_4$ ± 50 mM KIDA. Humidified air was continuously bubbled through the samples. After a 1-h equilibration period, 200 discs were placed in 20 ml fresh equilibration media and proton efflux was monitored at pH 6.5. Humidified, CO_2-free air was continuously bubbled through the incubation media. Rates of proton efflux were calculated from the linear slope of the titrant addition curves. Usually 30 min was required for the rate of efflux to reach linearity after addition of sucrose or K^+.

Electrical Measurements. A rectangular block of sugar beet tissue equilibrated in a 10-mM KIDA solution was snuggly fitted into a 57- × 7- × 7-mm^3 channel milled into a Lucite plate. Approximately 3 ml 10 mM KIDA solution (pH 7.0) used for tissue equilibration was pipetted into the channel so that the exposed surfaces of the tissue were covered with solution. A solution change to one of higher sucrose concentration was accomplished by simultaneously draining the 10 mM KIDA solution and adding 40 mM sucrose in 10 mM KIDA (pH 7.0). During the solution change, the tissue remained covered with liquid. To establish that the membrane depolarization was not due to tissue perturbations, solution changes in the absence of sucrose were used as controls. Membrane potentials were recorded by inserting the microelectrodes into sucrose-storing cells located in the ray and interzone tissue (unpublished data). Electrical recordings of penetrated tissue were not considered if: (*a*) the resistance (and associated tip potential) of the microelectrode increased during the course of the experiment; (*b*) the tip potential calibrations did not match each other within 2 mv; (*c*) the microelectrode penetrated adjacent to or into a nucleus; and (*d*) the penetrated cell collapsed.

RESULTS AND DISCUSSION

Effect of Sucrose Addition on Membrane Potential. For higher plants, as well as for most organisms, there is considerable evidence for the transport of sugars with cations (1, 15). If the co-transport process leads to a net movement of electric charge across the membrane, the membrane potential of those cells in which co-transport is occurring will be modified. Transient depolarizations have been observed upon addition of sugars to various higher plant tissues (ref. 12 and references therein). Initial electrical

[4] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[5] Abbreviations: KIDA, potassium iminodiacetic acid; Mops, morpholinopropanesulfonic acid; BTP, bis-Tris-propane; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

experiments in the root sink of sugar beets revealed that the membrane potential of sucrose-storing cells was about −64 mv from vacuole to ambient KIDA solution. On addition of sucrose-enriched KIDA, a membrane depolarization occurred followed by a spontaneous partial recovery (Fig. 1). Observed peak depolarizing responses ranged from 5 to 10 mv with an average of 8.2 ± 2.2 mv (10 trials on five interray and five interzone parenchymal cells). Depolarization took 1.2 ± 0.2 min, whereas repolarization lasted longer, 1.5 ± 0.1 min. The average repolarization was 7.7 ± 0.8 mv.

Effect of Protons on Sucrose Uptake. Since the electrical experiments were consistent with ionic co-transport of sucrose, specific ions were tested for their effect upon sucrose uptake. Evidence for proton co-transport of sugars in higher plant tissues is substantial. The findings supporting this theory include pH optima for sucrose uptake in the acidic range (pH 6.0–6.5) and declining rates of sucrose uptake as the pH is raised above 6.5. Figure 2 shows the effect of protons (pH) on sucrose uptake by discs of sugar beet roots. Sucrose uptake into the cytoplasm (2-h wash fraction) was totally passive (no CCCP effect) and was pH-independent. Although active sucrose transport (CCCP sensitive) into the vacuole was pH-dependent (pH optima of 6.5 ± 0.2, average of four experiments), passive sucrose uptake into the vacuole was insensitive to pH.

The direction and magnitude of the proton gradient across the tonoplast is difficult to determine in a complex tissue such as the sugar beet root. In an attempt to estimate vacuolar pH, 7 g tissue were homogenized in 25 ml 0.1 mM KCl (pH 6.8) for 30 s in a VirTis blender. The average pH of the homogenate was 6.24. Since the vacuole represents over 70% of the tissue volume, this pH is probably a reasonable estimate of the vacuolar pH. In most tissues, the vacuole is more acidic than the cytoplasm (16). Therefore, this estimated pH is probably higher than that existing in the intact tissue. If this estimate of the vacuolar pH is accurate, a proton gradient would exist from vacuole to free space at optimal media pH. The presence of such a gradient would provide evidence against a proton co-transport system at the tonoplast.

Effect of K^+ and Sucrose on Proton Efflux. When tissue discs were placed in deionized H_2O or 1 mM $CaSO_4$, the pH of the external media decreased to as low as 5.8 to 6.2. The rate of efflux was proportional to the external pH. Since the pH optima for sucrose uptake was 6.5, all proton efflux experiments were run at or near this pH.

The rate of net proton efflux varied greatly between experiments (different days and different roots), but the response to added K^+ and sucrose was consistent. Increasing the concentration of sucrose in the external media from 5 to 50 mM increased net proton efflux

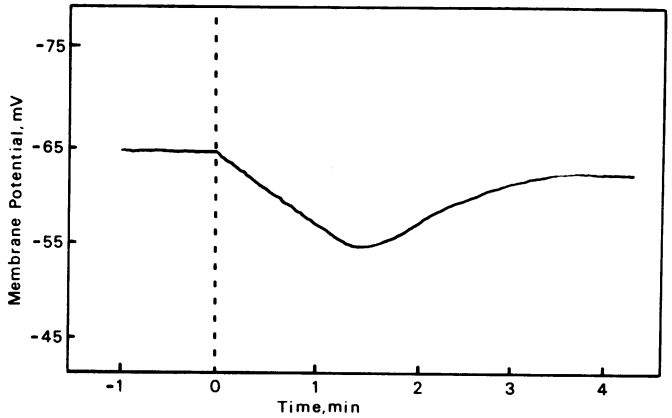

FIG. 1. Chart record showing the time course of sucrose-induced depolarization and spontaneous recovery of the membrane potential. Sucrose was increased 100 mM above the control solution at time zero (- - -).

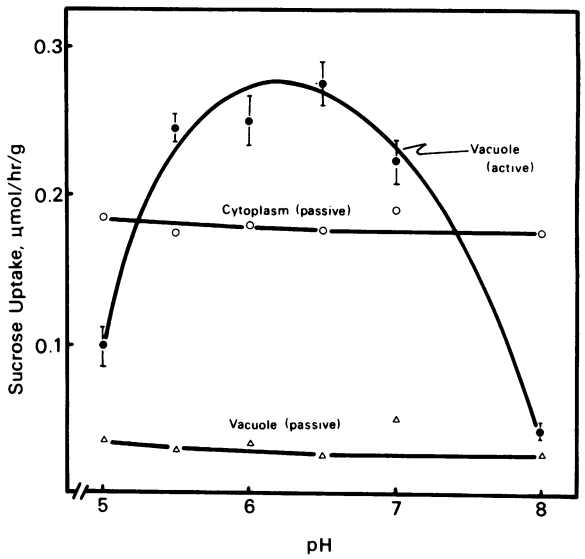

FIG. 2. pH effect on sucrose uptake and compartmentalization in sugar beet root tissue discs. Discs were washed for 30 min in running tapwater and then equilibrated for 1 h in 50 mM buffer at the appropriate pH, 1 mM $CaSO_4$, and 50 mM KIDA. The uptake media contained the equilibrium buffer solution plus 20 mM sucrose (30,000 dpm/µmol) ± 10 µM CCCP. After a 4-h uptake, the discs were rinsed three times for 3 min each in deionized H_2O to remove free space sucrose. This was followed by two 60-min washes to remove labeled sucrose from the cytoplasm. The discs then were extracted overnight in 80% ethanol to remove sucrose from the vacuole. Total dpm in a compartment in the absence of CCCP minus uptake in the presence of CCCP was considered as active uptake.

Table I. *Sucrose Effect on Proton Efflux by Root Tissue Discs*

Discs were washed for 30 min in running tapwater and then equilibrated for 60 min in 1 mM $CaCl_2$ with or without 50 mM KIDA. The equilibration media was replaced immediately prior to the determination of proton efflux with a pH-stat at pH 6.5.

Uptake Media (mM)	Proton Efflux	
	Experiment 1	Experiment 2
	nmol h^{-1} g^{-1}	
$CaCl_2$ (1)[a]	880	340
$CaCl_2$ (1) + sucrose (50)	973	440
$CaCl_2$ (1) + KIDA (50)	1213	
$CaCl_2$ (1) + KIDA (50)[a]	1053	520
$CaCl_2$ (1) + sucrose (50)	1227	660

[a] Also the equilibration media. Sequential additions of sucrose or KIDA were made after a 30-min linear rate had been established.

by an average of 96 nmol h^{-1} g^{-1} in the absence of added K^+ and 160 nmol h^{-1} g^{-1} in the presence of 50 mM KIDA (Table I). The addition of 50 mM KIDA enhanced net proton efflux 177 nmol h^{-1} g^{-1}. The ion-exchange properties of the pectinaceous cell walls in this tissue would provide substantial buffering capacity and exchange sites to trap the H^+ released. Therefore, the rates reported are no doubt conservative estimates of actual H^+ release rates.

Effect of K^+ and Na^+ Ions on Sucrose Uptake. Since the enhanced proton efflux with added sucrose was contrary to the theory of proton/sucrose co-transport, alternative cations were investigated for their effect on sucrose uptake. In animal cells and certain bacteria and fungi, sugars are co-transported with alkali cations (see references in ref. 1). Supplementing sucrose solutions with various concentrations of KIDA stimulated active uptake of

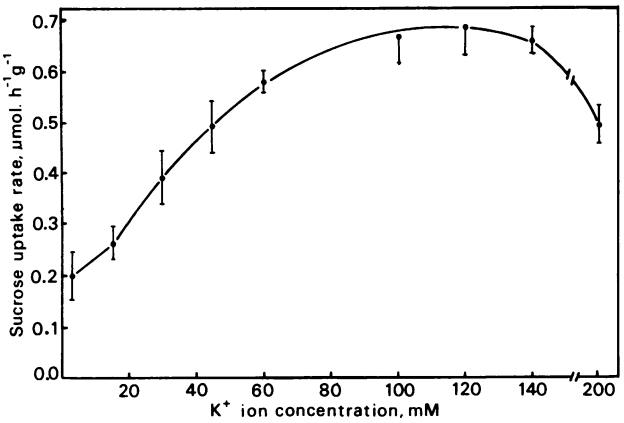

FIG. 3. Effect of K^+ ion concentration on sucrose uptake rate. The uptake solution contained 40 mM sucrose, 1 mM Mops (pH 6.5), and between 0 and 200 mM KIDA.

Table II. *Effect of Anions on Rate of Sucrose Uptake in Sugar Beet Root*

The uptake solution contained 40 mM sucrose, 1 mM Mops, and 100 mM anion (pH 6.5). Anions were supplied as BTP salts.

Anion Added	Sucrose Uptake		Uptake Inhibition
	Active	Passive	
	$\mu mol\ h^{-1}\ g^{-1}$		%
None	0.34 ± 0.03	0.13 ± 0.04	
Cl^-	0.18 ± 0.03	0.12 ± 0.02	48
NO_3^-	0.19 ± 0.02	0.12 ± 0.04	45
$HPO_4^{2-}/H_2PO_4^-$	0.25 ± 0.02	0.12 ± 0.02	27
SO_4^{2-}	0.25 ± 0.02	0.13 ± 0.02	27
Iminodiacetate[a]	0.34 ± 0.01	0.13 ± 0.02	1

[a] Iminodiacetate at pH 6.5 is a zwitterion with a net negative charge of about 1.

sucrose (Fig. 3). Maximum stimulation was observed at a concentration of 120 mM. Similar stimulations of sucrose uptake, with concentration optima between 100 and 120 mM K^+ ions, were observed when K^+ ions were supplied as KCl, KNO_3, K_2SO_4, or KH_2PO_4/K_2HPO_4. This stimulating effect of K^+ salts upon active uptake of sucrose corresponded to the activity of K^+ ions, with optimal uptake occurring at 95 mM activity. With optimal K^+ ion stimulation, active sucrose uptake was 2.1 and 4.0 times higher than control rates. The effect of Na salts on active uptake of sucrose was similar to, but somewhat greater than, that of K^+ salts (5–20%). This observed stimulation by alkali salts was not an osmotic effect of salt addition since similar osmotic modifications with BTP-Mops as the osmoticum did not influence active uptake of sucrose.

Effect of Anions on Sucrose Uptake. Although the stimulation of sucrose uptake by alkali salts correlated with the alkali-cation activities, and although anion/sugar co-transport has not, to the authors' knowledge, been reported in any organism, the effect of anions on sucrose uptake was tested in the absence of alkali cations to assure that the stimulation of sucrose uptake by alkali salts was, in fact, an alkali-cation effect. High concentrations of anions, supplied as BTP salts, inhibited rather than stimulated sucrose uptake (Table II). This inhibition correlated with the usual permeabilities of the anions across biological membranes, *i.e.* the more membrane-permeable anions had greater inhibitory effects on sucrose uptake.

Effect of Ionophores on Sucrose Uptake. The ionophores valinomycin, gramicidin D, and nigericin influence the transport of monovalent cations across biological membranes (see ref. 17 and references therein). Thus, these ionophores should influence sucrose uptake if sucrose transport is occurring by a cation/sucrose co-transport mechanism. Valinomycin, a highly specific ionophore that causes a dissipation of the K^+ ion electrochemical potential gradient, and gramicidin D, a rather nonspecific ionophore that dissipates K^+, Na^+, and proton gradients, strongly inhibited active uptake of sucrose (Table III). Nigericin, which specifically stimulates K^+ ion/proton exchange across membranes (17) reduced both active and passive uptake of sucrose; therefore, caution must be used in interpreting its specific effect on active sucrose transport.

Since valinomycin did not totally inhibit sucrose uptake in the previous experiment, it was unclear whether the inhibition was limited to K^+ enhanced sucrose uptake or whether there existed a valinomycin-insensitive fraction. Therefore, valinomycin was tested for its ability to inhibit sucrose uptake both in the presence and absence of added K^+. The results indicated that valinomycin inhibits sucrose uptake by 26% in the absence of K^+ and eliminates K^+ enhanced sucrose uptake (Table IV). Thus, a portion of the CCCP-sensitive active uptake is insensitive to valinomycin.

The transient membrane depolarization and proton fluxes observed upon exposure of root-sink cells to sucrose and the regulation of sucrose uptake by factors influencing the electrical and/or chemical potential gradients for protons and alkali cations

Table III. *Effect of Ionophores on Active Sucrose Uptake*

Discs were equilibrated for 90 min (30 discs/3 ml) in deionized H_2O. After equilibration, the discs were incubated in 3 ml 50 mM Mops (pH 6.5), 100 mM KIDA, 40 mM sucrose ± 10 μM CCCP. Ionophores were added in the concentrations shown.

Ionophore Treatment	Sucrose Uptake		Inhibition of Active Uptake
	Passive	Active	
μM	$nmol\ h^{-1}\ g^{-1}$		%
Control	483	217	
Valinomycin (1)	383	166	23
Valinomycin (10)	400	73	66
Gramicidin (1)	350	83	61
Gramicidin (10)	383	16	92
Nigericin (0.75)	283	138	36
Nigericin (7.5)	267	117	69
LSD (0.05)		38	

Table IV. *Valinomycin Inhibition of K-enhanced Sucrose Uptake into Vacuole of Root Tissue Discs*

Basal uptake media consisted of 50 mM Mops (pH 6.5), 1 mM $CaSO_4$, and 20 mM sucrose. Tissue discs (30 discs/3 ml) were preincubated for 1 h in the uptake media prior to the addition of [^{14}C]sucrose (30,000 dpm/μmol). After a 4-h incubation, the tissue was rinsed three times for 3 min each to remove free space label. This was followed by two 60-min washes. The combined 60-min washes represented label in the cytoplasmic compartment. The remaining label in the vacuole was extracted with 80% ethanol.

Uptake Media	Cytoplasm		Vacuole	
	Passive	Active	Passive	Active
	$nmol\ h^{-1}\ g^{-1}$			
Basal media	84 ± 7	0	107 ± 9	343 ± 17
Basal media + 50 mM K^+	79 ± 9	0	108 ± 18	517 ± 15
Basal media + 10 μM Valinomycin	85 ± 7	0	88 ± 9	252 ± 22
50 mM K^+ + 10 μM Valinomycin	80 ± 4	0	126 ± 27	284 ± 23

clearly suggested that sucrose is co-transported with ions in this tissue. Specific results supporting the conclusion that the ion involved is K^+ include: (*a*) the strong stimulation of sucrose uptake by K^+ (and Na^+); (*b*) the inhibition of sucrose uptake by the alkali cation ionophores valinomycin and gramicidin D; and (*c*) an apoplastic pH optimum above the apparent vacuolar pH. In addition, the use of a K^+-specific histochemical stain in this laboratory indicated that the pectinaceous cell wall, but not the vacuole, of the sucrose-storing root cells of sugar beet contained a high concentration of K^+.

The inverse relationship between the downhill electrochemical potential gradient for protons and sucrose transport in sugar beet root tissue is contrary to the proton co-transport mechanism of phloem loading suggested by Giaquinta (5) and Malek and Baker (13).

The theory that membrane transport of sugars is driven by a "proton motive force" generated as a result of proton pumping has received wide spread acceptance. Evidence for a proton-sucrose symport consistent with the theory is accumulating in a wide range of species and tissue types (1, 5, 8). However, many of these studies have not differentiated between transport at the plasmalemma and tonoplast (1, 8, 10, 15, 17), even though transport of sugars at the two membranes may be quite different. For example, although a −64 mv potential between vacuole and low K^+ external bathing media was found, it is generally held that the tonoplast potential is small and positive (16). Therefore, following accepted electrophysiological convention, the plasma membrane potential would be large and negative. Furthermore, the pH of the vacuole is lower than that of the cytoplasm. Therefore, the downhill electrochemical potential gradient for protons would be outward from the vacuole rather than inward. The results presented here would suggest that, in sugar beet root tissue, the downhill electrochemical potential gradient across the tonoplast is coupled with sucrose uptake via a proton antiport system and that sucrose transport is coupled to a K^+ gradient. Such a system for tonoplast transport has been suggested by Guy *et al.* (6) and may explain the results of Doll *et al.* (2), using isolated vacuoles. Doll *et al.* (2) showed an acidic pH optimum of 5.6 for isolated red beet vacuoles but also found a response to K^+ and Mg, presumably through stimulated ATPase activity. However, the pH of isolated vacuoles may increase during isolation (11). Changes in gradients during isolation of vacuoles makes comparative interpretation between them and tissue discs very difficult. However, it is conceivable that the carrier system may be responsive to proton gradients in both directions across the tonoplast, which would explain the discrepancy between the results presented here and those of Doll *et al.* (2).

Table V. *Characteristics Affecting Ionic Co-transport of Sucrose in Beet Root Tissue and Source Leaf Tissue in Certain Higher Plants*

Characteristics	Beet Root Tissue	Source Leaf Tissue	References[a]
Ionic			
Intracellular pH	6.0–6.5	8.0–8.5	7, 22
Effect of sucrose addition	Increase in net proton efflux	Decrease in net proton efflux	12
Vacuolar/cell wall K^+ ion concentration ratio	Low	High	
Sucrose uptake			
Optimal apoplastic pH	>6.5	<6.5	3, 12
Effect of K^+ ion	Stimulation	Inhibition	3

[a] References for source leaf tissue data.

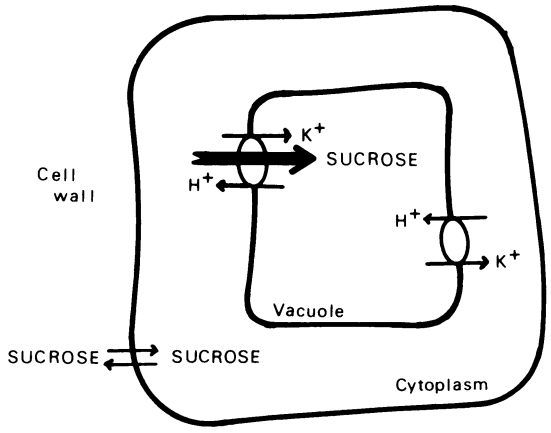

FIG. 4. A working model for sucrose transport in the root sink of sugarbeets. A tonoplast pump generates proton and K^+ ion gradients which drive sucrose uptake via a carrier protein with K ion/proton exchange capacity.

Characteristics of sucrose transport and cellular composition in the beet root sink contrasts in several ways with phloem loading of sucrose in source leaves (Table V). These contrasting characteristics led to the hypothesis that the proton and/or K^+ ion gradients which drive sucrose transport in higher plants occur in opposite directions in source and sink cells. It is proposed that the sucrose transport mechanisms in sink tissues are coupled to a proton/K^+ ion-exchange reaction, such as that shown in Figure 4, and it is envisioned that the energetic uphill movement of sucrose into the vacuole is coupled to the downhill movement of alkali cations into the vacuole and probably to the downhill movement of protons out of the vacuole. The potential gradients for alkali cations and protons may be generated and maintained by a vectorial alkali cation/proton exchange "pump" which would prevent the long-term buildup of alkali cations within the cell. Evidence for involvement of a vectorial membrane ATPase in the pump mechanism is not clear. However, our preliminary data indicate that ATPase inhibitors, with the exception of oligomycin, inhibit sucrose uptake in discs of sugar beet roots.

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