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In Vitro Analysis of the H^+-Hexose Symporter on the Plasma Membrane of Sugarbeets (*Beta vulgaris* L.)[1]

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ABSTRACT

The mechanism of hexose transport into plasma membrane vesicles isolated from mature sugarbeet leaves (*Beta vulgaris* L.) was investigated. The initial rate of glucose uptake into the vesicles was stimulated approximately fivefold by imposing a transmembrane pH gradient (ΔpH), alkaline inside, and approximately fourfold by a negative membrane potential (Δψ), generated as a K^+-diffusion potential, negative inside. The -fold stimulation was directly related to the relative ΔpH or Δψ gradient imposed, which were determined by the uptake of acetate or tetraphenylphosphonium, respectively. Δψ- and ΔpH-dependent glucose uptake showed saturation kinetics with a K_m of 286 micromolar for glucose. Other hexose molecules (e.g. 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and D-mannose) were also accumulated into plasma membrane vesicles in a ΔpH-dependent manner. Inhibition constants of a number of compounds for glucose uptake were determined. Effective inhibitors of glucose uptake included: 3-*O*-methyl-D-glucose, 5-thio-D-glucose, D-fructose, D-galactose, and D-mannose, but not 1-*O*-methyl-D-glucose, D- and L-xylose, L-glucose, D-ribose, and L-sorbose. Under all conditions of proton motive force magnitude and glucose and sucrose concentration tested, there was no effect of sucrose on glucose uptake. Thus, hexose transport on the sugarbeet leaf plasma membrane was by a H^+-hexose symporter, and the carrier and possibly the energy source were not shared by the plasma membrane H^+-sucrose symporter.

The transport of sugars across the plasma membrane of plant cells provides carbon and energy required for growth. Numerous studies of glucose uptake into intact tissues or cells suggest that uptake is carrier-mediated, although in some cases the mechanism of uptake is still unclear. Beginning with the investigations in *Chlorella*, the presence of a glucose transporter, which utilizes the energy of the proton motive force to drive the accumulation of glucose, was demonstrated in plants (13). Subsequent to that report, other examples of H^+-mediated glucose uptake in higher plants have been reported (15, 16).

In animal as in plant cells, the oxidation of glucose represents a major source of energy. In general, there are two types of glucose carriers in mammals: a Na^+-glucose cotransporter and a facilitated carrier (2). The cotransporter is a glycoprotein of 664 amino acids and is expressed in epithelial cells of the small intestine and kidney. Its nucleotide sequence has homology with the Na^+-proline cotransporter from *Escherichia coli* (11) and, thus, it belongs to a family of symport proteins that transport H^+ or Na^+ and sugar, amino acid, or iodide molecules (27).

Five isoforms of the mammalian-facilitated glucose carrier have been identified and are designated GLUT1 through 5 (2). The proteins are also glycosylated and are composed of 492 to 524 amino acids. From 39 to 65% of these amino acids groups are conserved in all isoforms, with 50 to 76% representing conservative substitutions (20). The mammalian-facilitated transporter is homologous to facilitated carriers for glucose, maltose, and galactose in yeast (6, 25) and xylose- and arabinose-H^+ symporters in *E. coli* (1). Each of the five isoforms is differentially expressed in tissues, and activity of one of these isoforms (GLUT4) is increased in response to insulin, exercise, and feeding (22). The mechanism of regulation seems to be through the redistribution of carriers to the plasma membrane (22) and/or posttranslational modification of the carrier at the plasma membrane (10), and not gene activation.

The genes for the hexose-H^+ symporter of *Chlorella* and its homolog in *Arabidopsis* have been cloned and sequenced (18, 19). The translated and transcribed products of these genes confer hexose transport activity when transformed into a heterologous system, *Schizosaccharomyces pombe*; thus, there can be little doubt that they encode the hexose-H^+ symporter (17, 18). In *Arabidopsis*, the protein consists of 522 amino acids, and the gene shows structural homology with the H^+-glucose symporter in *Chlorella*, a H^+-arabinose symporter in *E. coli*, and the facilitated glucose transporter in human, rat, and yeast (19).

Despite the relative importance of glucose as an energy and carbon source in higher plants, relatively few investigations have been conducted on the biochemical aspects of the hexose carrier on the plasma membrane (for reviews see 14–16). Apart from the glucose uptake studies in intact tissues, there are a limited number of studies of uptake in cells or membrane vesicles. Getz *et al.* (7), for example, clearly demonstrated a glucose transport system on the plasma membrane of beet root protoplasts. Uptake was specific for glucose in that fructose did not inhibit glucose uptake. Interestingly, the rate of glucose uptake was affected by tissue differentiation. Similar developmental-related effects on glucose uptake in *Cucurbita* and *Pelargonium* have been noted (cited in ref. 14) and may indicate some form of regulation of the glucose uptake activity.

Stanzel *et al.* (23, 24) investigated sugar uptake into *Streptanthus* suspension cells. At sugar concentrations of <1 mM, their data indicated two transport systems, one for glucose

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with a low transport capacity for fructose and another with approximately equal affinities for glucose and fructose. At high sugar concentrations (>10 mM), there was a linear, nonsaturating uptake of a number of structurally unrelated sugars (24). The mechanism and pathway of this low-affinity but high-capacity uptake was not determined. One of the more extensive studies on glucose uptake in suspension-cultured plant cells of *Chenopodium* was conducted by Gogarten and Bentrup (8, 9). Using exchange-diffusion analyses, a detailed study of the catalytic mechanism of transport and the characteristics was conducted.

Isolated membrane vesicles represent a somewhat simplified system for studying membrane transport processes, which in the case of glucose transport has not been widely used in plants. In only one study has glucose uptake been investigated on membrane fractions with known composition (26). In that study, glucose transport activities were analyzed in tonoplast and plasma membrane fractions, and the uptake activities could be clearly distinguished based on their catalytic properties, substrate specificity, and sensitivity to sulfhydryl reagents. Uptake of glucose was only slightly stimulated by the presence of a ΔpH,[2] alkaline inside, which might indicate the presence of a facilitated glucose transporter similar to that found in mammalian cells. In this report, we have employed a plasma membrane vesicle system from sugarbeet (*Beta vulgaris* L.) leaves, which has previously been used to analyze a H^+-sucrose symporter (4), to investigate glucose uptake. We report here an analysis of a plasma membrane H^+-hexose symporter.

MATERIALS AND METHODS

Plant Material and Isolation of Plasma Membrane

Sugarbeets (*Beta vulgaris* L. cv Tina EE; Kleinwanzlebener Saatzucht, Göttingen, FRG) were grown in hydroponic culture as described by Bush (5). Fully expanded, basal leaves from 56- to 168-d-old-plants were harvested 1 to 3 h after the beginning of the light period, and plasma membrane vesicles were isolated by aqueous two-phase partitioning (4). The final plasma membrane fraction was typically diluted in 5 mM Hepes-Btp, pH 7.5, buffer containing 1 mM KCl and 350 mM sorbitol (dilution buffer) and concentrated by centrifugation (50,000*g* for 60 min; SS34 rotor, Du Pont de Nemours, Bad Homburg, FRG). When plasma membrane vesicles were preloaded with K^+, the K^+ concentration was increased to 50 mM or as indicated in the text. Protein concentration was determined by the method of Bradford (3).

Assay for ΔpH- and Δψ-Dependent Hexose Uptake

Hexose uptake was determined by the filtration method described by Buckhout (4). ΔpH-dependent uptake was defined as the difference in hexose uptake in vesicles diluted into dilution buffer, titrated to pH 6.0 with Mes, in the absence and presence of 2 μM CCCP. CCCP was dissolved in ethanol, and assays conducted in the absence of CCCP contained an equal volume of ethanol. The hexose incorporation was determined at 0.5, 1, 1.5, 2, and 4 min or as indicated, and the hexose uptake rate was determined from these data by linear regression analysis. Other conditions are stated in the legends to the figures.

For Δψ-dependent glucose uptake, plasma membrane vesicles were diluted approximately 100-fold in dilution buffer (see above) containing 50 mM K_2SO_4, concentrated by centrifugation as above, resuspended in dilution buffer at a protein concentration of 10 to 15 $mg \cdot mL^{-1}$ and frozen at −70°C. Plasma membranes were thawed and then used for transport. Transport assays were initiated by diluting the plasma membrane approximately 50-fold in dilution buffer containing 50 mM Na_2SO_4 instead of K_2SO_4, glucose at the desired concentration, and 1 μM valinomycin. At the desired time interval, 100-μL samples were taken, the plasma membranes were collected on nitrocellulose filters (0.22 μm), and the filters were washed with ice-cold dilution buffer as for ΔpH-dependent glucose uptake. Incorporated activity was determined by liquid scintillation counting.

Analysis of K_i

The effects of hexose analogs on glucose uptake were tested as follows. Analogs were dissolved in dilution buffer at a final concentration of 25 mM. ΔpH-dependent glucose uptake was determined as above in the presence of 25 μM glucose and 100, 200, 400, 600, and 800 μM analog. Data were plotted by the method employed by Hitz *et al.* (12). The basis for this analysis is as follows. For Michaelis-Menten kinetics:

$$V_o = \frac{(V_{max}S)}{K_m + S} \quad (1)$$

where V_o is the reaction rate at substrate concentration, *S*. V_{max} and K_m have their usual meaning. In the presence of a competitive inhibitor, the rate can be described as:

$$V_i = \frac{(V_{max}S)}{K_m\left(1 + \frac{I}{K_i}\right) + S} \quad (2)$$

where V_i is the reaction rate at substrate concentration, *S*, and inhibitor concentration, *I*. It follows then that

$$\frac{V_o}{V_i} = \frac{K_m + \frac{K_m}{K_i}(I) + S}{K_m + S} \quad (3)$$

and for $K_m \gg S$

$$\frac{V_o}{V_i} = 1 + \frac{1}{K_i}(I) \quad (4)$$

Thus, a plot of $\frac{V_o}{V_i}$ *versus* *I* yields a line with a slope of $\frac{I}{K_i}$.

This method of K_i determination assumes competitive inhibition, *i.e.* the V_{max} remains unaltered. We have determined V_{max} for glucose uptake in the presence of D-galactose and 3-*O*-methyl-D-glucose and found them to be statistically similar to V_{max} for glucose alone. K_i values were determined graphi-

[2] Abbreviations: ΔpH, transmembrane pH gradient; Δψ, transmembrane electrical potential; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; K_i, inhibition constant; TPP^+, tetraphenylphosphonium.

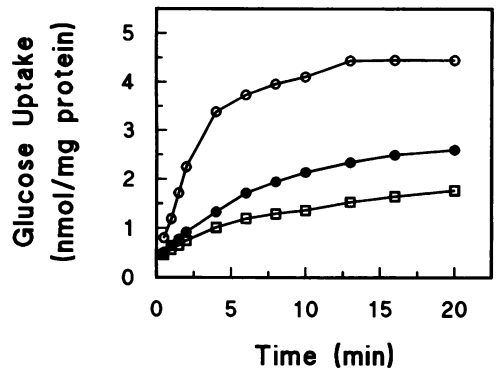

Figure 1. ΔpH-dependent glucose uptake into plasma membrane vesicles. Plasma membrane vesicles were equilibrated at pH 7.5 and diluted approximately 50-fold into pH 7.5 (●), pH 6.0 (○), or pH 6.0 buffer containing 2 μM CCCP (□) and 100 μM glucose. Results are an average of three independent determinations.

cally from the intersection of the lines. In all cases, lines were drawn using linear regression analysis and the intersections calculated mathematically.

Assay for Acetic Acid and TPP+ Uptake

Uptake of [^{14}C]acetic acid was conducted essentially as described (4, 5). Nitrocellulose filters (0.22 μm) were used to collect the vesicles. The assay contained 20 μL of plasma membrane vesicles (approximately 1.0 mg protein) and 980 μL of buffer (350 mM sorbitol, 5 mM Hepes-Btp, pH 7.5 or adjusted to pH 6.0 with Mes and 5.5 μM acetic acid, 0.3 μCi). Valinomycin (1 μM) was also included to prevent trapping of acetic acid (5) and to maintain conditions similar to the ΔpH-dependent hexose uptake assay. CCCP (2 μM) was added to demonstrate that the accumulated acetate was not bound to the vesicle membrane.

The uptake of [^{3}H]TPP+ was conducted as for acetate uptake but with 20 μL of plasma membrane (approximately 10 mg protein) and 980 μL of dilution buffer containing valinomycin and K_2SO_4 or Na_2SO_4 as indicated. In control experiments, valinomycin was replaced by a similar amount of ethanol. The final concentration of [TTP+] was 44 μM (1 nCi/mL). To avoid excessive binding of TPP+ to filter material, cellulose acetate filters (0.22 μm pore size) were used.

Determination of Vesicle Volume

To determine vesicle volumes [^{14}C]inulin (0.1 μCi carrier-free) and ^3H_2O (3.5 μCi) were added to 25-μL plasma membranes (300 μg protein) suspended in 970 μL dilution buffer and incubated for 10 min at 10°C. Vesicles were pelleted for 20 min at 100,000*g* and radioactivity was determined in pellets and supernatants. The volume was calculated according to Bush (5) and found to be 4 ± 1 $\mu L \cdot mg^{-1}$ protein.

Materials

Unless otherwise stated, the sources for chemicals are described elsewhere (4, 21). Cellulose acetate membrane filters were purchased from Schleicher and Schuell (Düsseldorf, FRG) and [^{3}H]TTP+ from New England Nuclear (Boston, MA).

RESULTS

ΔpH-Dependent Glucose Uptake in Plasma Membrane Vesicles

When plasma membrane vesicles that had been equilibrated at pH 7.5 were diluted 50-fold into isoosmotic buffer at pH 6.0, a ΔpH-dependent uptake of glucose was observed (Fig. 1). ΔpH-dependent glucose uptake was linear for approximately 2 min and decreased to zero after approximately 20 min. The initial rate of uptake was fivefold greater than that for plasma membrane vesicles diluted into pH 7.5 buffer or into pH 6.0 buffer containing 2 μM CCCP, a protonophore. The initial rate and the final extent of ΔpH-dependent glucose uptake was positively correlated with the magnitude of the imposed ΔpH gradient (Fig. 2A). The presence of a ΔpH was confirmed by the uptake of the weak acid of acetate (Fig. 3A). Acetate accumulated in vesicles equilibrated at pH 7.5

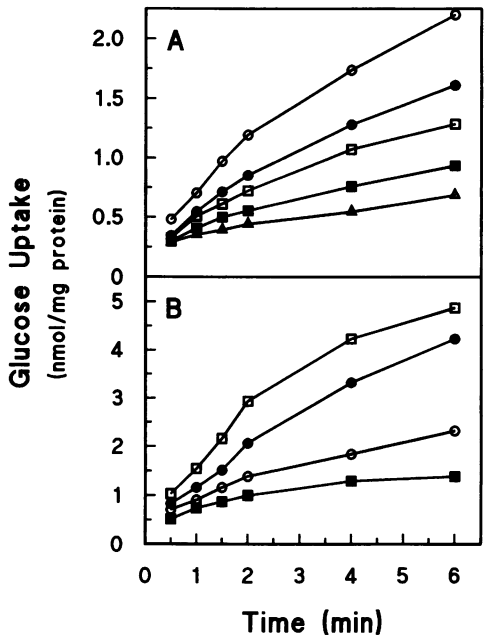

Figure 2. Analysis of ΔpH- and Δψ-dependent glucose uptake in plasma membrane vesicles. A, Plasma membrane vesicles were equilibrated at pH 7.5 and diluted 50-fold into buffer that had been adjusted to pH 6.0 (○), 7.07 (●), 7.3 (□), 7.5 (■), or 6.0 containing 2 μM CCCP (Δ). Uptake of glucose was determined at the indicated time points as described in "Materials and Methods." Results are the average of two independent determinations. B, Δψ-Dependent glucose uptake was determined by equilibrating vesicles at pH 7.5 in the presence of 100 mM K_2SO_4 and 1 μM valinomycin. Equilibrated vesicles were diluted approximately 50-fold into pH 7.5 buffer containing 100 mM K_2SO_4 (ΔK^+ = 0, ■); 75 mM K_2SO_4 and 25 mM Na_2SO_4 (ΔK^+ = 50, ○); 50 mM K_2SO_4 and 50 mM Na_2SO_4 (ΔK^+ = 100 mM, ●); or 100 mM Na_2SO_4 (ΔK^+ = 200 mM, □). Uptake was determined at the time points indicated as described in "Materials and Methods." Results are the average of two independent determinations.

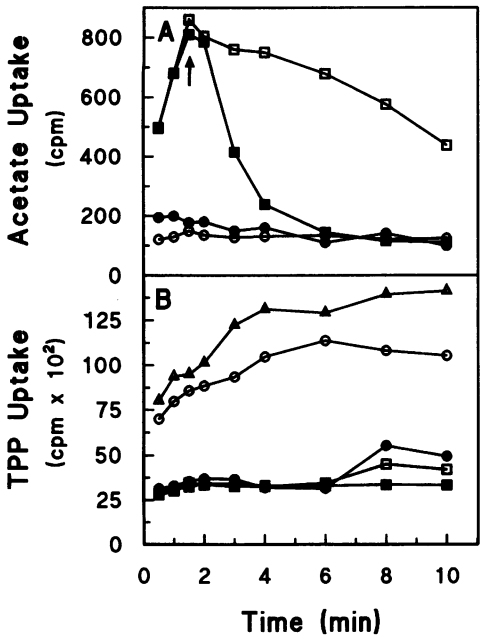

Figure 3. Accumulation of acetate and TPP^+ in plasma membrane vesicles. A, Acetate uptake was determined in plasma membrane vesicles equilibrated at pH 7.5 and diluted into buffer at pH 6.0 (□), pH 7.5 (○), pH 6.0 containing 2 μM CCCP (●), or pH 6.0 with 2 μM CCCP added at the time point indicated by the arrow (■). Results are the average of three independent determinations. B, TPP^+ uptake was determined in vesicles equilibrated in 50 mM or in one case 100 mM K_2SO_4 (△) and diluted approximately 50-fold into pH 7.5 buffer containing: 50 mM Na_2SO_4 in the presence (○) or absence (●) of 1 μM valinomycin; 50 mM K_2SO_4 and valinomycin at pH 7.5 (□) or 6.0 (■); or 100 mM Na_2SO_4 and valinomycin (△). Results are the average of two independent determinations.

and diluted into pH 6.0 buffer, and this accumulation was reversed by the protonophore, CCCP, which is consistent with the assumption that a ΔpH was formed.

In the experiment shown in Figure 1, 1 μM valinomycin was present, which maintained the $\Delta\psi$ at or near zero and prevented the inhibition of glucose uptake through an increasing, positive membrane potential. The resulting transport equilibrium established at approximately 10 min was then likely a result of an internal glucose concentration against which no net glucose uptake occurred. Uptake of glucose showed saturation, Michaelis-Menten-like kinetics with respect to glucose concentration with a K_m of 0.29 ± 0.07 mM (Fig. 4). The passive glucose uptake, determined as uptake at pH 6.0 but in the presence of CCCP, was consistently lower than uptake into vesicles equilibrated at pH 7.5 and diluted into pH 7.5 buffer (Fig. 1). The K_m value for active glucose uptake were also determined using the pH 7.5 passive uptake values. This determination resulted in a K_m of 0.12 ± 0.1 mM (data not shown) and was not significantly different (greater than 2 SD from the mean) from the K_m determined in Figure 4. Therefore, subsequent determinations of passive glucose uptake were conducted at pH 6 in the presence of CCCP.

For an active transport process, the concentration of glucose in the vesicle at equilibrium should exceed that of the external solution. The vesicle volume, determined with [^{14}C] inulin to calculate the external volume and ^3H_2O to calculate total pellet volume (5), was 4 ± 1 $\mu L \cdot mg^{-1}$ protein. Using this volume and the data in Figure 1, one can calculate that the concentration of glucose within the vesicles was 5.5 times greater than the external glucose concentration. Thus, the uptake occurred against a concentration gradient.

Δψ-Dependent Glucose Uptake in Plasma Membrane Vesicles

The above data are consistent with a H^+-glucose symport mechanism. With such a symporter, the movement of H^+ produces a charge separation and, thus, the overall transport process is electrogenic. To test the validity of this assumption, the effect of a membrane potential, generated as a K^+ diffusion potential, on glucose uptake was tested. The relative magnitude of $\Delta\psi$ was varied by equilibrating vesicles in buffer containing 50 mM K_2SO_4 and diluting them in isoosmotic buffer containing various concentrations of K_2SO_4 and Na_2SO_4 so that their combined concentration was 50 mM. Increasing the Na^+ concentration from 1 to 50, 100, and 200 mM resulted in a concomitant increase in glucose uptake activity by 1.3-, 1.9-, and 3.2-fold, respectively (Fig. 2B). Vesicles diluted into 200 mM Na^+ were preincubated in buffer containing 200 mM K^+. Thus, this uptake was dependent on $\Delta\psi$. The presence of a $\Delta\psi$, negative inside, was demonstrated by the accumulation of TPP^+ under the conditions used to determine the $\Delta\psi$-dependent glucose uptake (Fig. 3B). The accumulation of TPP^+ was dependent on valinomycin and the presence and magnitude of a K^+ concentration gradient. Thus, the presence of a $\Delta\psi$ was demonstrated.

Substrate Specificity of the H^+-Hexose Symporter

In other organisms, the H^+-glucose symporter transports a number of hexoses and is more accurately described as a hexose symporter (14). Therefore, the ΔpH-dependent up-

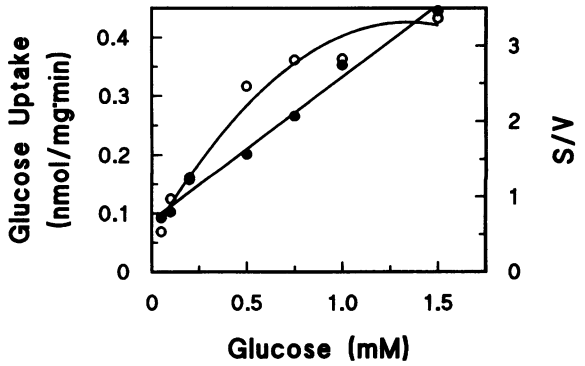

Figure 4. Kinetic analysis of ΔpH-dependent glucose uptake into plasma membrane vesicles. The rate of ΔpH-dependent glucose uptake was determined at the indicated glucose concentration as described in "Materials and Methods" (○). The data were analyzed by the method of Wolfe-Hanes and a K_m of 286 ± 68 μM and V_{max} of 0.453 $nmol \cdot (min \cdot mg)^{-1}$ were determined (●). Results are the average of three independent determinations.

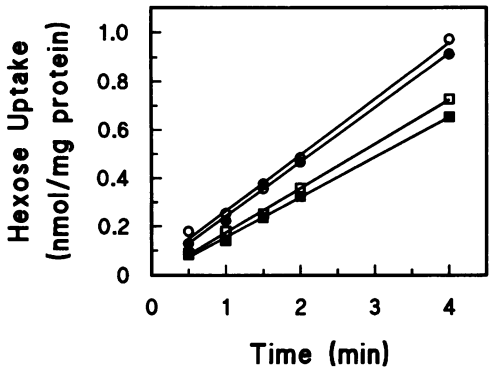

Figure 5. Analysis of ΔpH-dependent uptake of glucose, glucose analogs, and mannose into plasma membrane vesicles. ΔpH-dependent uptake was determined in the presence of 0.1 mM D-glucose (○), 2-deoxy-D-glucose (●), 3-*O*-methyl-D-glucose (□), or D-mannose (■) as described in "Materials and Methods." The uptake rate in the absence of a pH gradient was subtracted from uptake in the presence of a gradient to obtain the illustrated data. Results are the average of three independent determinations.

take of three hexose molecules, 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and D-mannose, was tested. The rate of the ΔpH-dependent uptake of 2-deoxy-D-glucose was statistically similar to that of D-glucose, whereas the rate of 3-*O*-methyl-D-glucose and D-mannose uptake was approximately 25% reduced compared with the rate for D-glucose (Fig. 5). In addition, hexose analogs were tested for their ability to inhibit ΔpH-dependent glucose uptake. Of the analogs tested, 5-thio-D-glucose, 3-*O*-methyl-D-glucose, D-galactose, D-mannose, and D-fructose all inhibited glucose uptake with K_i values in the approximate range of the K_m value for glucose

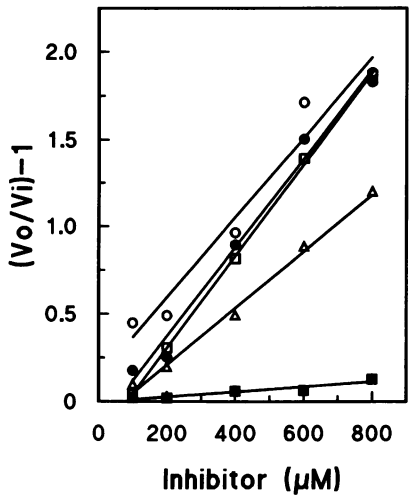

Figure 6. Determination of K_i values for various hexoses on glucose uptake. ΔpH-dependent glucose uptake was determined in the presence of 25 μM glucose (*i.e.* glucose concentration ≪ than the K_m) in the presence of the indicated concentrations of 3-*O*-methyl-D-glucose (○), D-galactose (●), D-mannose (□), L-glucose (■), or D-fructose (△). The K_i values were determined as described in "Materials and Methods." Slopes of the resulting linear curves were determined by linear regression analysis. Values are the average of two independent determinations.

Table I. *Substrate Specificity of the H+-Hexose Symporter*

ΔpH-dependent glucose uptake was determined in the presence of 25 μM glucose and 100, 200, 400, 600, and 800 μM analog. The K_i values were calculated as described in "Materials and Methods." The K_m for glucose was 0.29 mM. Values are the average of three determinations.

Analog	K_i
	mM
D-Mannose	0.38
D-Galactose	0.39
3-*O*-Methyl-D-glucose	0.44
5-Thio-D-glucose	0.55
D-Fructose	0.62
L-Sorbose	1.29
D-Xylose	1.93
L-Xylose	3.19
D-Ribose	4.65
L-Glucose	6.78
1-*O*-Methyl-D-glucose	6.89

uptake (Fig. 6, Table I). D-Ribose, L-sorbose, 1-*O*-methyl-D-glucose, D- and L-xylose, and L-glucose all had K_i values of greater than 10 times that of the K_m for glucose and were considered to be weak substrates for the hexose carrier. From these data, it can be reasonably concluded that on the sugarbeet plasma membrane the glucose carrier is more generally a H+-hexose symporter.

Competition between Sucrose and Glucose Uptake

The same plasma membrane preparation that catalyzes ΔpH-dependent hexose uptake also catalyzes ΔpH-dependent sucrose uptake (4). In an initial attempt to determine if sucrose and glucose were accumulated in the same or different vesicles, we have attempted to find uptake conditions in which sucrose inhibited the uptake of glucose. However, in no case was an effect of sucrose on glucose uptake (Fig. 7A) or an effect of glucose on sucrose uptake (Fig. 7B) found. This was true for the initial rate of glucose or sucrose uptake and also the equilibrium concentrations of both sugars (data not shown). Also, in experiments in which the proton motive force was decreased so as to produce a relatively low rate of glucose uptake, similar to the rate shown in Figure 2A for a ΔpH of 0.2, no effect of sucrose on glucose uptake was observed. Even when the buffer capacity of the dilution buffer was reduced to 0.5 mM Hepes, there was no effect of sucrose on glucose uptake (data not shown). Under no condition was glucose uptake influenced by sucrose, and the possibility exists that the H+-hexose and the H+-sucrose symporters are spatially segregated in unique vesicles. Yet, the possibility still remains that even at the lowest imposed proton motive force there was sufficient energy to drive uptake of sucrose and glucose to equilibrium in a single vesicle.

DISCUSSION

The data presented clearly demonstrate the active uptake of glucose into plasma membranes of sugarbeet leaf vesicles.

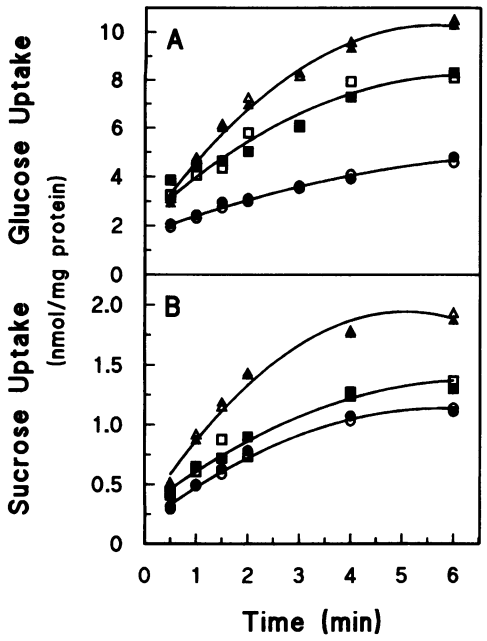

Figure 7. Effect of sucrose concentrations of ΔpH-dependent glucose uptake and the effect of glucose concentrations on ΔpH-dependent sucrose uptake. ΔpH-dependent glucose and sucrose uptake was determined as described in "Materials and Methods" or by Buckhout (4), respectively. A, ΔpH-dependent glucose uptake was determined at 0.5 (○, ●), 1.5 (□, ■), and 2.5 mM (△, ▲) glucose in the presence (open symbols) or absence (closed symbols) of 2 mM sucrose. Results are the average of two independent determinations. B, Analysis of ΔpH-dependent sucrose uptake determined at 0.1 (○, ●), 0.5 (□, ■), and 1 mM (△, ▲) sucrose in the presence (open symbols) or absence (closed symbols) of 1 mM glucose. Results are the average of two independent determinations.

The arguments supporting the active and/or carrier-mediated uptake of glucose are: (a) uptake in the presence of ΔpH and/or $\Delta\psi$, (b) the accumulation of glucose against a concentration gradient, and (c) the substrate specificity of the transport process. In a formal sense, these data are also consistent with a OH^--glucose antiporter; however, H^+ are transported by the plasma membrane ATPase, and therefore it seemed more likely that the accumulation of glucose is driven by the proton motive force and not by a hydroxyl ion gradient.

Previous investigations from our laboratory have shown that glucose was not recognized by the sucrose symport carrier (4) and that glucose did not induce a transient alkalization, an observation that is inconsistent with the presence of a H^+-glucose cotransporter in the plasma membrane fraction used by Slone and Buckhout (21). The data presented in this report contradict those of Slone and Buckhout (21), because plasma membrane vesicles clearly accumulated glucose in a manner consistent with the activity of a H^+-hexose symporter. The resolution of this inconsistency is at the moment not apparent; however, the variety of sugarbeet used by Slone and Buckhout (21), Mono HYE 4, and that used in this study, Tina EE, may be important in this regard. It may be the case that the glucose uptake activity in the Mono HYE 4 variety was either below detectability of the alkalization assay as suggested by Slone and Buckhout (21) or that the activity in Mono HYE 4 was labile. These possibilities are currently under investigation.

The substrate specificity of the symporter showed that the carrier not only recognized the glucose molecules but also several other hexose molecules. As with glucose carrier on the plasma membranes of *Chlorella* (14) and *Chenopodium* (9) cells, the hexose carrier transported a variety of pyranose molecules (see below). In sugarbeet, the hexose carrier clearly preferred the D-stereoisomer over the L-stereoisomer but showed no preference of equatorial *versus* axial orientation for the hydroxyl groups at position C2, because mannose and 2-deoxyglucose were transported (Fig. 5). Also, the equatorial *versus* axial orientation for the hydroxyl groups at position C4 was likely unimportant in recognition because D-galactose inhibited glucose uptake in a competitive manner with a K_i approximately equal to the K_m for glucose uptake (Table I). Furthermore, the binding at the C3 position of the pyranose ring could not have been particularly tight because 3-*O*-methyl-D-glucose was transported at only a slightly lower rate than the parent compound, glucose (Fig. 5). From the analysis of substrate specificity in *Chenopodium*, the equatorial orientation of the hydroxyl group at C3 was important because D-allose did not inhibit glucose uptake (9). The binding of glucose to the carrier at C1 of the pyranose ring showed a distinct preference for the free hydroxyl because the 1-*O*-methyl-D-glucose was a weak inhibitor of glucose uptake.

Somewhat surprising then was the ability of fructose to inhibit glucose uptake with a K_i approximately twice that of the K_m for glucose transport (Table I). Although no data were presented for actual transport of fructose in this system, the inhibitory activity suggested an interaction of fructose with the hexose carrier. Fructose also inhibited glucose uptake in *Chenopodium* (9) and *Streptanthus* (23) cells, although no fructose inhibition of glucose uptake was detected in beetroot protoplasts (7) or tobacco cells (26).

In summary, the substrate specificity of the hexose carrier in *Beta* resembled closely that found in *Chenopodium*, which considering the phylogenetic relationship of the two genera was not surprising. There was, however, one difference: D-xylose inhibited glucose uptake in *Chenopodium* but not in *Beta*. Thus, there might be some unique features of the *Beta* hexose carrier at C6 that distinguishes it from the carrier in *Chenopodium*. In general, these data demonstrate the presence of a H^+-hexose symporter on the plasma membranes of sugarbeet leaves, and they provide a basis for the further biochemical investigation of this activity.

ACKNOWLEDGMENT

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