Plant Cell Physiol. 38(8): 910–916 (1997)

Characterization of Hexose Transporter for Facilitated Diffusion of the Tonoplast Vesicles from Pear Fruit

Katsuhiro Shiratake [1], Yoshinori Kanayama [2] and Shohei Yamaki [3]

Laboratory of Horticultural Science, School of Agricultural Sciences, Nagoya University, Chikusa, Nagoya, 464-01 Japan

Tonoplast vesicles were prepared from the flesh tissue of mature pear fruit. Sugar uptakes into the vesicles determined by two different methods, the membrane and the gel filtration methods, were quite similar. The uptake was highest for glucose and subsequently, in order, for fructose, sucrose and sorbitol. It was not stimulated by addition of ATP, although the vesicles could create a proton gradient. However, the uptakes were significantly inhibited by *p*-chloromercuribenzene sulphonate (PCMBS, SH-reagent and inhibitor of sugar transporter). Further, the PCMBS-sensitive uptakes of glucose and fructose saturated with their increased concentrations. Thus, these PCMBS-sensitive uptakes are mediated by the transporter of facilitated diffusion. The uptakes of glucose or fructose each had two K_m values. K_m values for glucose were 0.35 and 18 mM, and those for fructose were 1.6 and 25 mM. The uptake of 0.2 mM glucose was inhibited by 2 mM fructose and that of 2 mM fructose was inhibited by 2 mM glucose, but neither was inhibited by sucrose or sorbitol. *O*-methyl-glucose (OMG) also inhibited both the glucose and fructose uptakes. Therefore, the same transporter may mediate both glucose and fructose uptakes at lower concentrations; this hexose transport system differed from the sucrose and sorbitol transport systems.

Key words: Facilitated diffusion — Fructose — Glucose — Pear (*Pyrus communis*) fruit — Sugar transport — Tonoplast vesicles.

Sugar is a primary and important storage compound in some plant tissues, such as many fruits, beet root, and sugarcane stalk. The vacuole is the major cellular compartment for sugar accumulation in such sink tissues. Therefore, it is very important to clarify the mechanism of sugar transport across the tonoplast. The sugar transport mechanism of the plant plasma membrane has been studied at the molecular level (Buckhout and Tubbe 1996, Tanner and Caspari 1996). Genes for the sucrose symporter and glucose transporter have been isolated from some plant species. Further studies of histochemistry and transgenic plants by the antisense method have demonstrated that the sucrose symporter plays an important role in phloem loading of source leaves. On the other hand, the transport mechanism of sugar across the tonoplast has been studied far less than that of plasma membrane, although it is considered to function effectively in sugar accumulation of sink tissue.

Beet root and sugarcane stalk are typical sugar storage tissues; their major accumulated sugar is sucrose. Some reports showed a proton/sucrose antiport mechanism in the tonoplast of sugar beet (Doll et al. 1979, Getz 1991) and red beet roots (Briskin et al. 1985b). Getz et al. (1991) reported that a proton/sucrose antiport mechanism also existed in the tonoplast of sugarcane stalk. In contrast, other reports showed only facilitated diffusion of sucrose in the sugarcane tonoplast (Preisser and Komor 1991, Williams et al. 1990). In the tonoplasts of sucrose storage tissues, both active transport and facilitated diffusion of sucrose have been reported; and the mechanism of such sucrose accumulation is still controversial.

Another typical sugar storage tissue is fruit; some of them accumulate hexose as a primary storage sugar. In Rosaceae plants such as pear and apple, part of the photoassimilate is synthesized to sorbitol, which is unloaded into fruit with sucrose. Then the sorbitol and sucrose are converted into hexose and accumulate in the fruit (Berüter 1985, Loescher and Everard 1996, Yamaki and Ino 1992). Yamaki et al. (1993) reported the hexose is the major accumulated sugar in both the cytosol and vacuole of the pear fruit cell. Therefore, a hexose transport mechanism might exist in the tonoplast of pear fruit. Yamaki (1987) reported the active transports of sorbitol and sucrose into the vacuoles isolated from immature apple fruit. However, it is still unclear whether a hexose transporter exists in the tonoplast of pear fruit and how sugars accumulate in the vacuoles. In tomato fruit, hexose is also the major storage sugar, although the primary translocated sugar of tomato plants is sucrose. Milner et al. (1995) reported distinct transporters of facilitated diffusion for glucose and fructose existed in the tonoplast vesicles from tomato fruit. In this study, we observed a relatively high activity of facilitated diffusional transport for glucose and fructose in the tonoplast vesicles

Abbreviations: BSA, bovine serum albumin; BTP, bis-tris propane; DTT, dithiothreitol; OMG, *O*-methyl-glucose; PCMBS, *p*-chloromercuribenzene sulphonate.

[1] Research Fellow of the Japan Society for the Promotion of Science.

[2] Present address: Faculty of Agriculture, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, 981 Japan.

[3] To whom correspondence should be addressed.

from pear fruit. Interestingly, the details of this hexose transport were considerably different from those of tomato fruit.

Materials and Methods

Plant material—Pear (*Pyrus communis* L. var. *sativa* DC, cv. 'La France') fruits were harvested at maturity (middle of October) and stored at −1°C.

Isolation of tonoplast vesicles—Tonoplast vesicles were isolated by a modification of the methods described previously (Hosaka et al. 1994, Maeshima and Yoshida 1989). The flesh tissue of pear fruit was grated in 70 mM Tris-HCl buffer (pH 8.0) containing 250 mM mannitol, 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'* tetraacetic acid (EGTA), 0.5% (w/v) BSA (fraction V powder), 4 mM DTT, 12.5 mM β-mercaptoethanol, 5% (w/v) polyvinylpyrrolidone (PVP-40), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM $MgSO_4$ and homogenized with a grass homogenizer. The homogenate was centrifuged at 10,000×*g* for 20 min. The supernatant was centrifuged at 100,000×*g* for 30 min. The precipitate was suspended in the suspension medium (250 mM mannitol, 1 mM DTT, 2 mM $MgSO_4$ and 4 mM BTP-MES, pH 7.2) and sonicated for 20 s. After the suspension was centrifuged at 10,000×*g* for 20 min, the supernatant was centrifuged at 100,000×*g* for 30 min. This microsomal precipitate was suspended in 4 mM BTP-MES buffer (pH 7.2) containing 500 mM sucrose, 1 mM DTT, and 2 mM $MgSO_4$ and poured into a centrifugation tube. The suspension was overlayered with the suspension medium. After centrifugation at 100,000×*g* for 1 h with RPS27-2 rotor (Hitachi, Tokyo, Japan), the interface layer was collected and diluted with the suspension medium. The suspension was then centrifuged at 125,000×*g* for 30 min. The precipitate was washed with the suspension medium and centrifuged again. The precipitate was suspended in a small volume of the suspension medium and used as tonoplast vesicles within one day.

ATPase and proton transport activities—The activities of ATPase and proton transport were measured by modifications of the methods of Matsuura-Endo et al. (1990) and Briskin et al. (1985a), respectively. The ATP hydrolysis activity was colorimetrically measured by determining the rates of liberation of P_i at 30°C. The standard reaction mixture for ATPase activity contained 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM KCl, 1 mM sodium molybdate, 0.02% Triton X-100, and 50 mM BTP-MES (pH 7.5). Proton transport activity was measured at 20°C as the quenching rate of fluorescence of quinacrine. The standard reaction medium contained 250 mM mannitol, 50 mM KCl, 5 μM quinacrine, 3 mM Tris-ATP, 3 mM $MgSO_4$, and 20 mM BTP-MES (pH 7.5). The change in fluorescence was monitored with a fluorescence spectrophotometer (Hitachi 850) set at 430 nm for excitation and at 500 nm for emission.

Sugar transport activity—Sugar uptake was assayed by the membrane filtration method in most experiments and by the gel filtration method in some cases. All procedures were carried out at 20–25°C. Sugar uptake by the membrane filtration method was assayed by a modification of the method of Getz (1991). Vesicles (60–100 μg protein) were preincubated in 0.9 ml of the reaction medium (278 mM mannitol, 3 mM $MgSO_4$, 50 mM KCl, ±3 mM Tris-ATP, ±2.22 mM PCMBS and 4 mM BTP-MES, pH 6.5) for 10 min. Uptake was initiated by addition of 0.1 ml [^{14}C]-sugar solution (370–1,480 kBq ml^{-1}). 120 μl aliquot of reaction mixture was diluted with 1.3 ml of the washing buffer (250 mM mannitol, 45 mM KCl and 4 mM BTP-MES, pH 6.5) to stop the sugar uptake at each interval. The diluted reaction mixture was filtered under vacuum through a cellulose nitrate filter (0.45 μm pore size, Millipore) pre-wetted with the washing buffer. The filter was washed 4 times with 1 ml of the washing buffer. The radioactivity retained on the filter was determined by liquid scintillation spectrometry. The gel filtration method was performed by a modification of the method of Moriyama et al. (1993). Sephadex G-50 (fine, Pharmacia) was pre-equilibrated with the washing buffer. The 1 ml syringe column filled with the gel was centrifuged at 100×*g* for 2 min in a swing-out rotor. The 150 μl aliquots of reaction mixture were taken at intervals, immediately applied to the column, and centrifuged at 100×*g* for 2 min. The radioactivity contained in the eluate was determined by liquid scintillation spectrometry. Total uptake and PCMBS-insensitive uptake of sugar were determined by the reactions without and with 2 mM PCMBS, respectively. PCMBS-sensitive uptake of sugar was calculated by subtraction of PCMBS-insensitive uptake from total uptake.

Protein assay—Protein content was determined by the method of Bradford (1976), using BSA as a standard.

Results

ATPase and proton transport activities of tonoplast vesicles—ATPase activity of tonoplast vesicles was inhibited to 10 and 34% of the control by nitrate and bafilomycin (V-type ATPase inhibitors), respectively. Vanadate (P-type ATPase inhibitor) reduced ATPase activity slightly, and azide (F-type ATPase inhibitor) had little effect (Table 1). The ability of the vesicles to establish a proton gradient was determined by measuring ionophore-reversible quenching. As shown in Fig. 1, approximately 80% of proton transport activity was inhibited by nitrate. The degree of inhibition corresponded closely to that of ATPase activity.

Effect of ATP and PCMBS on sugar uptake into tonoplast vesicles measured by two different methods—We applied two different methods for determination of sugar uptake into the tonoplast vesicles: the membrane filtration and gel filtration methods. When assayed by the membrane filtration method, glucose, fructose, sucrose and sorbitol were taken up into the tonoplast vesicles (Table 2). The

Table 1 Inhibitor sensitivity of ATP hydrolysis activity associated with tonoplast vesicles

Inhibitor	ATP hydrolysis	
	μmol P_i (mg protein)$^{-1}$ h^{-1}	% (control)
Control	17.7	100
KNO_3 (150 mM)	1.84	10
Na_3VO_4 (100 μM)	14.5	82
Bafilomycin (100 nM)	6.03	34
NaN_3 (1 mM)	16.7	94

ATPase activity was determined as described in Materials and Methods.

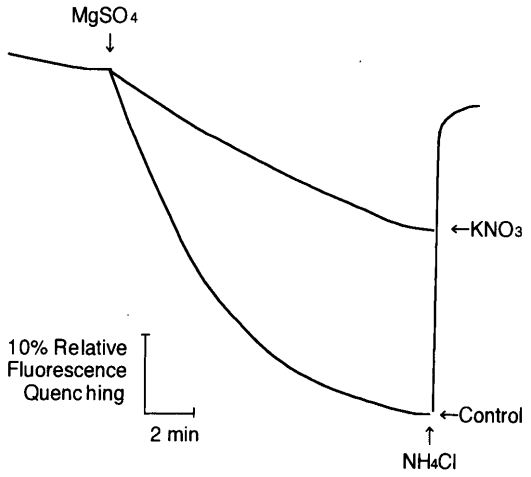

Fig. 1 ATP-dependent quenching of quinacrine fluorescence in tonoplast vesicles. Final concentration of KNO_3 was 150 mM. The activities of ATP-dependent proton transport in the absence and presence of KNO_3 were 46.9 and 12.5 percent fluorescence min^{-1} $(mg\ protein)^{-1}$, respectively.

uptake was highest for glucose and, subsequently, in order for fructose, sucrose, and sorbitol. The uptakes of these four sugars were significantly inhibited by PCMBS (PCMBS-sensitive uptake); this result suggests that their uptakes were mediated by a transporter. In particular, the uptakes of glucose and fructose were inhibited to 18 and 35% of the control by PCMBS, respectively; the inhibition of sucrose and sorbitol uptakes were about 40%. The uptakes of these four sugars were not activated by the addition of ATP.

The uptake of each sugar was also examined by the gel filtration method to verify the lack of stimulation after adding ATP (Table 2). The uptake of each sugar was quite similar to that by the membrane filtration method; uptakes of glucose and fructose were large and inhibited predominantly by PCMBS, while those of sucrose and sorbitol were

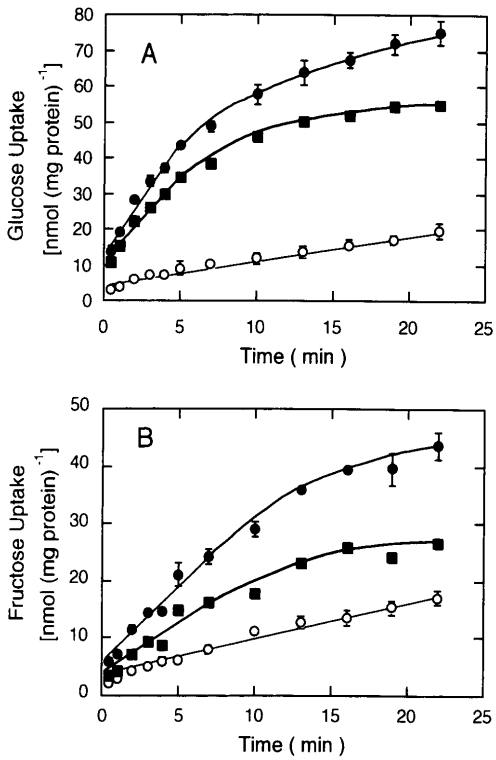

Fig. 2 Time courses of 1 mM glucose and fructose uptakes into tonoplast vesicles. A, glucose uptake. B, fructose uptake. Total uptake, PCMBS-sensitive uptake, and PCMBS-insensitive uptake were expressed as ●, ■ and ○, respectively. Sugar uptake was measured by the membrane filtration method.

smaller. Uptakes of these four sugars were never stimulated by ATP. By two different methods, we obtained almost the same results. The uptakes not inhibited by PCMBS (PCMBS-insensitive uptake) were not very different among these four sugars and were considered to be simple diffusions. The glucose and fructose uptakes were steady enough to characterize; on the other hand, sucrose and sor-

Table 2 Effect of ATP and PCMBS on uptake of each sugar compared between the membrane filtration and the gel filtration methods

Treatment	Sugar uptake [nmol $(mg\ protein)^{-1}$ min^{-1}]							
	Glucose	%	Fructose	%	Sucrose	%	Sorbitol	%
Membrane Filtration								
Control	2.63±0.04	(100)	1.30±0.11	(100)	0.50±0.03	(100)	0.38±0.04	(100)
ATP	2.51±0.05	(95)	1.07±0.07	(82)	0.46±0.04	(92)	0.37±0.04	(97)
PCMBS	0.48±0.05	(18)	0.44±0.04	(34)	0.28±0.03	(56)	0.23±0.02	(61)
Gel Filtration								
Control	4.71±0.19	(100)	1.85±0.07	(100)	0.67±0.09	(100)	0.47±0.03	(100)
ATP	4.30±0.10	(91)	1.67±0.07	(90)	0.72±0.05	(107)	0.46±0.00	(97)
PCMBS	0.36±0.01	(8)	0.34±0.03	(18)	0.35±0.03	(52)	0.31±0.01	(66)

Final concentration of each sugar was 1 mM. Different tonoplast vesicles were prepared for the membrane and the gel filtration methods.

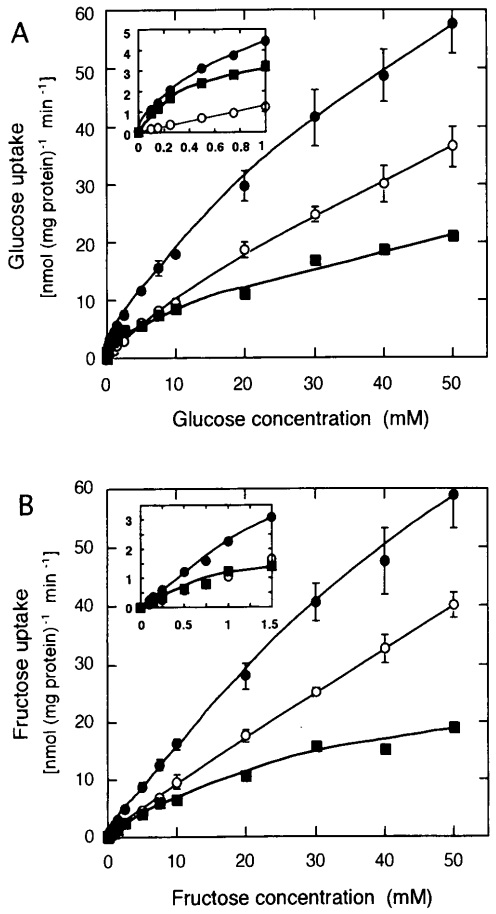

Fig. 3 Concentration dependence of glucose and fructose uptakes into tonoplast vesicles. A, glucose uptake. B, fructose uptake. Total uptake, PCMBS-sensitive uptake, and PCMBS-insensitive uptake were expressed as ●, ■ and ○, respectively. Inset of each graph shows the magnification of the low concentration range. Sugar uptake was measured by the membrane filtration method.

bitol uptakes were somewhat erratic. Next we further characterized the glucose and fructose uptakes.

Kinetics of hexose uptake—Time courses of glucose and fructose uptakes are shown in Fig. 2. PCMBS-sensitive uptake of glucose was initially rapid, with a large proportion of the uptake occurring before the first sampling time (30 s). Similar inclinations were observed in the sucrose uptake into the tonoplast vesicles from tomato fruit (Milner et al. 1995) and sugarcane stalk (Williams et al. 1990). The uptake then increased almost linearly with time for first several minutes, but the uptake rate slowed with prolonged reaction. PCMBS-sensitive uptake of fructose showed the almost same pattern as that of glucose. PCMBS-insensitive uptake of glucose or fructose was also initially rapid but then continued to increase linearly with time for 20 min. This result also suggested that the PCMBS-insensitive uptake is a simple diffusion rather than a carrier-mediated one. In further experiments, the incubation time for sugar uptake was determined within 10 min.

The concentration dependent curves of glucose and fructose uptakes are shown in Fig. 3. PCMBS-sensitive uptake of glucose was comprised of two components which were concentration dependent, while PCMBS-insensitive uptake rose linearly in proportion to glucose concentration (Fig. 3A). The presence of the two component system was shown more clearly by Lineweaber-Burk plots of PCMBS-sensitive uptake. The K_m values of components in the lower and higher glucose concentrations were estimated to be 0.35 and 18 mM, respectively (Fig. 4A, B). The curve of PCMBS-sensitive uptake of fructose was also comprised of two components in both a low and high fructose concentration (Fig. 3B). The K_m values of the components were estimated to be 1.6 and 25 mM (Fig. 4C, D).

Table 3 Competition between 0.2 mM glucose or 2 mM fructose uptake into tonoplast vesicles and other sugars

Treatment	(mM)	0.2 mM glucose uptake		Treatment	(mM)	2 mM fructose uptake	
		nmol (mg protein)$^{-1}$ min^{-1}	% of control			nmol (mg protein)$^{-1}$ min^{-1}	% of control
Control		1.53	100	Control		1.88	100
Fructose	0.2	1.38	90	Glucose	0.2	1.63	87
	2	0.87	57		2	1.06	56
	20	0.29	19		20	0.34	18
OMG	0.2	1.40	92	OMG	0.2	1.65	88
	2	0.77	50		2	1.51	80
	20	0.15	10		20	0.58	31
Sucrose	2	1.66	108	Sucrose	2	1.80	96
	20	1.62	106		20	1.73	92
Sorbitol	2	1.44	94	Sorbitol	2	1.63	87
	20	1.60	105		20	1.95	104

Sugar uptake was measured by the membrane filtration method.

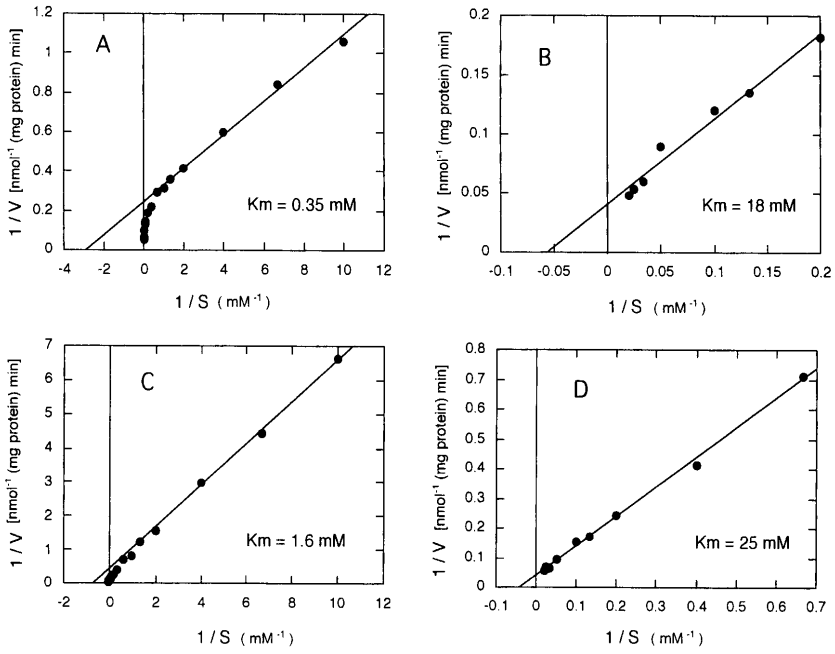

Fig. 4 Lineweaver-Burk plots of PCMBS-sensitive uptakes of glucose and fructose. Data came from the same experiment shown in Fig. 3. A and C show uptakes of glucose and fructose, respectively. B or D shows the magnification of the higher concentration range of A or C, respectively.

Competition for PCMBS-sensitive hexose uptake with each sugar—The competition for PCMBS-sensitive hexose uptake with each sugar was examined for the components at the lower concentration of glucose (K_m=0.35 mM) or fructose (K_m=1.6 mM). As shown in Table 3, the uptake of 0.2 mM glucose was reduced to 57 and 19% of the control by addition of 2 and 20 mM fructose, respectively, and to 50 and 10% of the control by addition of 2 and 20 mM OMG, respectively. The uptake of 2 mM fructose was also inhibited to 56 and 18% of the control by addition of 2 and 20 mM glucose, respectively, and to 31% of the control by addition of 20 mM OMG (Table 3). However, the uptakes of both sugars were not inhibited by the addition of either sucrose or sorbitol.

Discussion

To characterize the tonoplast vesicles from pear fruit, the activities of ATP hydrolysis and ATP-dependent proton-transport were determined with some inhibitors for ATPases. ATPase activity of the tonoplast vesicles was inhibited substantially by nitrate and bafilomycin (Table 1). ATP-dependent proton transport, detected as ionophore-reversible quenching, was also inhibited by nitrate (Fig. 1). Vanadate slightly inhibited ATPase activities of the tonoplast vesicles (Table 1). When we separated microsomes from pear fruit by continuous sucrose density gradient centrifugation, the highly tonoplast rich fraction also contained vanadate sensitive ATPase activity at the same level as in the present data (data not shown). Some reports have shown that ATP-dependent pumps inhibited by vanadate exist in tonoplasts, for example, proton pumps (Magnin et al. 1995, Müller et al. 1996), calcium pumps (Fukumoto and Venis 1986, Pfeiffer and Hager 1993), and a glutathione *S*-conjugate pump (Martinoia et al. 1993). Therefore, the vanadate sensitive ATPase activities of pear tonoplast might originate in the tonoplast rather than be caused by contamination by the plasma membrane. We concluded that our tonoplast vesicles contained little contamination by other membranes and were sealed to create a proton gradient across the tonoplast.

As assayed by the membrane filtration method, the uptake into the tonoplast vesicles was highest for glucose and, subsequently, in order for fructose, sucrose, and sorbitol. The uptakes were significantly inhibited by PCMBS but not stimulated by the addition of ATP (Table 2). Therefore the uptake of each sugar was also examined by the gel filtration method, which has the advantage of using enough more vesicles compared with the membrane filtration method to detect fainter changes in uptake. The uptakes of these four sugars by this method followed the same pattern; they were significantly inhibited by PCMBS and never stimulated by ATP (Table 2). Thus, the results of the sugar uptakes by two different methods coincided, indicating that the present results are reliable and reflect the reality of the sugar transport in these isolated tonoplast vesicles. PCMBS-sensitive uptakes of glucose and fructose saturated with their increased concentrations (Fig. 3). From the

above results, it was indicated that a transporter for facilitated diffusions of glucose and fructose exists in the tonoplast vesicles. In pear and apple fruit, hexose is a primary storage sugar (Berüter 1985, Yamaki and Ino 1992). In more detail, Yamaki et al. (1993) reported hexose is the major accumulated sugar in both the cytosol and vacuole of pear fruit cell. Thus it is reasonable that relatively high hexose transport was detected in the tonoplast of pear fruit. The hexose transporter may contribute the process of sugar accumulation into vacuoles and/or the export of sugar from vacuoles. Hexose transport in the tonoplast, especially in hexose storage tissue, has been studied in far less detail than those of the plasma membrane and sucrose transport of the tonoplast. Therefore, we further characterized this hexose transport.

The concentration dependent curves of PCMBS-sensitive uptakes of glucose and fructose were comprised of two components at low and high concentrations. Such a two component system was reported in the sucrose uptake into the tonoplast vesicles from red beet root (Getz 1991) and sugarcane stalk (Getz et al. 1991), in hexose uptake into the vacuoles from barley mesophyll (Martinoia et al. 1987), and in hexose uptake into the tonoplast vesicles from tobacco cells (Verstappen et al. 1991). Although such two components systems of sugar transport have been observed not only in the tonoplast but also in the plasma membrane of some plant species (Buckhout and Tubbe 1996), it is still unclear whether there are two different transporters or a single transporter responding differently to low and high hexose concentrations. It is suggested that isolated tonoplast vesicles might include both right side out and inside out vesicles and that the results of sugar transport might derive from both sides of these vesicles. There is some possibility that a single transporter mediates both import into vacuoles and export from vacuoles and has different affinities for the different directions, resembling the asymmetric transporter reported for erythrocyte hexose transport (Wheeler and Hinkle 1985).

To determine the substrate specificity of hexose transport, competition for the uptake of the lower glucose or fructose K_m values was studied. The uptake of 0.2 mM glucose competed with fructose, and the uptake of 2 mM fructose competed with glucose (Table 3). Further, both the uptakes of 0.2 mM glucose and 2 mM fructose competed with OMG. These results suggested that the uptakes of glucose and fructose at the lower concentrations were mediated by the same transporter. Sucrose and sorbitol did not inhibit the uptakes of glucose and fructose (Table 3). Therefore, this hexose transport system differs from the sucrose and sorbitol transport systems. OMG uptake into the tonoplast vesicles from tobacco cell was inhibited by glucose and fructose (Verstappen et al. 1991). Martinoia et al. (1987) suggested that fructose uptake into the vacuoles from barley mesophyll was mediated by the glucose transport system. The concentration dependencies of these hexose uptakes in tobacco and barley tonoplast also showed as two component systems and relatively low K_m values. Thus these hexose transport systems might be similar to that of pear fruit. In contrast, Milner et al. (1995) suggested that distinct transporters mediated glucose and fructose uptakes into the tonoplast vesicles of tomato fruit, and their concentration dependent curves were comprised of only one component. Their K_m values for hexose transport were approximately 120 mM, much higher than those of pear fruit. Therefore the hexose transport system of tomato fruit tonoplast may differ from that of pear fruit, although both pear and tomato fruit are hexose storage tissues. To clarify the hexose transport mechanism of tonoplasts, including the behavior of two component systems and the differences among plant species, further investigation is necessary.

At present, there is no information concerning the hexose transporter of the tonoplast at the protein and molecular levels. Sucrose transporters have only been partially purified from the tonoplast of sugarcane (Getz et al. 1994, Thom et al. 1992) and red beet (Getz et al. 1993). Recently, a putative cDNA encoded tonoplast sugar transporter was screened from a cDNA library of sugar beet, although its function is not clear (Chiou and Bush 1996). Further biochemical studies, such as the isolation of transporters and their genes, may lead to clarification of the sugar transport mechanism of tonoplasts.

We thank Mr. K. Sakakibara, research technician of the Nagoya University, for growing the pears. This work was supported in part by Grant-in-Aids (No. 07456018, 09306002 and 90001692) from the Ministry of Education, Science and Culture of Japan.

References

Berüter, J. (1985) Sugar accumulation and changes in the activities of related enzymes during development of the apple fruit. *J. Plant Physiol.* 121: 331–341.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.

Briskin, D.P., Thornley, W.R. and Wyse, R.E. (1985a) Membrane transport in isolated vesicles from sugarbeet taproot. I. Isolation and characterization of energy-dependent, H^+-transporting. *Plant Physiol.* 78: 865–870.

Briskin, D.P., Thornley, W.R. and Wyse, R.E. (1985b) Membrane transport in isolated vesicles from sugarbeet taproot. II. Evidence for a sucrose/H^+-antiport. *Plant Physiol.* 78: 871–875.

Buckhout, T.J. and Tubbe, A. (1996) Structure, mechanisms of catalysis, and regulation of sugar transporters in plants. *In* Photoassimilate Distribution in Plants and Crops. Edited by Zamski, E. and Schaffer, A.A. pp. 229–260. Marcel Dekker, Inc., New York.

Chiou, T.J. and Bush, D.R. (1996) Molecular cloning, immunochemical localization to the vacuole, and expression in transgenic yeast and tobacco of a putative sugar transporter from sugar beet. *Plant Physiol.* 110: 511–520.

Doll, S., Rodier, F. and Willenbrink, J. (1979) Accumulation of sucrose in vacuoles isolated from red beet tissue. *Planta* 144: 407–411.

Fukumoto, M. and Venis, M.A. (1986) ATP-dependent Ca^{2+} transport in

tonoplast vesicles from apple fruit. *Plant Cell Physiol.* 27: 491–497.
Getz, H.P. (1991) Sucrose transport in tonoplast vesicles of red beet roots is linked to ATP hydrolysis. *Planta* 185: 261–268.
Getz, H.P., Grosclaude, J., Kurkdjian, A., Lelièvre, F., Maretzki, A. and Guern, J. (1993) Immunological evidence for the existence of a carrier protein for sucrose transport in tonoplast vesicles from red beet (*Beta vulgaris* L.) root storage tissue. *Plant Physiol.* 102: 751–760.
Getz, H.P., Thom, M. and Maretzki, A. (1991) Proton and sucrose transport in isolated tonoplast vesicles from sugarcane stalk tissue. *Physiol. Plant.* 83: 404–410.
Getz, H.P., Thom, M. and Maretzki, A. (1994) Monoclonal antibodies as tools for the identification of the tonoplast sucrose carrier from sugarcane stalk tissue. *J. Plant Physiol.* 144: 525–532.
Hosaka, M., Kanayama, Y., Shiratake, K. and Yamaki, S. (1994) Tonoplast H^+-ATPase of mature pear fruit. *Phytochemistry* 36: 565–567.
Loescher, W.H. and Everard, J.D. (1996) Sugar alcohol metabolism in sinks and sources. *In* Photoassimilate Distribution in Plants and Crops. Edited by Zamski, E. and Schaffer, A.A. pp. 185–207. Marcel Dekker, Inc., New York.
Maeshima, M. and Yoshida, S. (1989) Purification and properties of vacuolar membrane proton-translocating inorganic pyrophosphatase from mung bean. *J. Biol. Chem.* 264: 20068–20073.
Magnin, T., Fraichard, A., Trossat, C. and Pugin, A. (1995) The tonoplast H^+-ATPase of *Acer pseudoplatanus* is a vacuolar-type ATPase that operates with a phosphoenzyme intermediate. *Plant Physiol.* 109: 285–292.
Martinoia, E., Grill, E., Tommasini, R., Kreuz, K. and Amrhein, N. (1993) ATP-dependent glutathione S-conjugate 'export' pump in the vacuolar membrane of plants. *Nature* 364: 247–249.
Martinoia, E., Kaiser, G., Schramm, M.J. and Heber, U. (1987) Sugar transport across the plasmalemma and the tonoplast of barley mesophyll protoplasts. Evidence for different transport systems. *J. Plant Physiol.* 131: 467–478.
Matsuura-Endo, C., Maeshima, M. and Yoshida, S. (1990) Subunit composition of vacuolar membrane H^+-ATPase from mung bean. *Eur. J. Biochem.* 187: 745–751.
Milner, I.D., Ho, L.C. and Hall, J.L. (1995) Properties of proton and sugar transport at the tonoplast of tomato (*Lycopersicon esculentum*) fruit. *Physiol. Plant.* 94: 399–410.
Moriyama, Y., Tsai, H.L. and Futai, M. (1993) Energy-dependent accumulation of neuron blockers causes selective inhibition of neurotransmitter uptake by brain synaptic vesicles. *Arch. Biochem. Biophys.* 305: 278–281.
Müller, M.L., Irkens-Kiesecker, U., Rubinstein, B. and Taiz, L. (1996) On the mechanism of hyperacidification in lemon. Comparison of the vacuolar H^+-ATPase activities of fruits and epicotyls. *J. Biol. Chem.* 271: 1916–1924.
Pfeiffer, W. and Hager, A. (1993) A Ca^{2+}-ATPase and a Mg^{2+}/H^+-antiporter are present on tonoplast membranes from roots of *Zea mays* L. *Planta* 191: 377–385.
Preisser, J. and Komor, E. (1991) Sucrose uptake into vacuoles of sugarcane suspension cells. *Planta* 186: 109–114.
Tanner, W. and Caspari, T. (1996) Membrane transport carriers. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 595–626.
Thom, M., Getz, H.P. and Maretzki, A. (1992) Purification of a tonoplast polypeptide with sucrose transport properties. *Physiol. Plant.* 86: 104–114.
Verstappen, R., Ranostaj, S. and Rausch, T. (1991) The hexose transporters at the plasma membrane and the tonoplast of transformed plant cells: kinetic characterization of two distinct carriers. *Biochim. Biophys. Acta* 1073: 366–373.
Wheeler, T.J. and Hinkle, P.C. (1985) The glucose transporter of mammalian cells. *Annu. Rev. Physiol.* 47: 503–517.
Williams, L., Thom, M. and Maretzki, A. (1990) Characterization of a proton translocating ATPase and sucrose uptake in a tonoplast-enriched vesicle fraction from sugarcane. *Physiol. Plant.* 80: 169–176.
Yamaki, S. (1987) ATP-promoted sorbitol transport into vacuoles isolated from apple fruit. *Plant Cell Physiol.* 28: 557–564.
Yamaki, S. and Ino, M. (1992) Alteration of cellular compartmentation and membrane permeability to sugars in immature and mature apple fruit. *J. Amer. Soc. Hort. Sci.* 117: 951–954.
Yamaki, S., Ino, M., Ozaki, S., Tsuchimoto, Y. and Ofosu-Anim, J. (1993) Cellular compartmentation and transport into tonoplast vesicles of sugars with ripening of pear fruit. *Acta Hort.* 343: 12–17.

(Received January 17, 1997; Accepted May 13, 1997)