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Different Mass Transfer Rates of Labeled Sugars and Tritiated Water in Xylem Vessels and Their Dependency on Metabolism

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ABSTRACT

Solutions of ^{14}C-sugars in tritiated water or solutions of ^{14}C- and ^{3}H-sugars were perfused by gravity through the xylem vessels of excised tomato internodes (*Lycopersicon esculentum*) mostly during 2 hours.

Mass flow of a solution in plant vessels is found not to be in conflict with different mass transfer rates of sugar and water molecules. It is explained by individual lateral escape rates from the vessel for each single compound. The escape of tritiated water can be ascribed to diffusion, while the escape of sugars is apparently linked to the metabolism of the surrounding parenchyma cells (Q_{10} sucrose uptake = 3.5). Lateral escape rates of sugars from the xylem vessels are in the proportion of sucrose-glucose-fructose (2.4:1.0:0.6). The accessibility of xylem parenchyma cells and their differential permeability to sugars control the longitudinal mass transfer of sugars in the xylem vessels.

As sucrose and glucose do not compete for uptake from the vessels by the contiguous cells, separate uptake systems for both may be postulated.

When labeled sugars, dissolved in tritiated H_2O, are administered to leaves or petioles, sugars are always transported more rapidly than tritiated H_2O in the phloem (1, 5, 7, 9, 20). Experiments with bark strips of Salix (16) gave similar results. Some authors, therefore, concluded that mass flow is an unlikely (7, 9) or even impossible (15, 16) transport mechanism. Others have given preference to the mass flow concept (1, 5, 20).

The xylem vessels of excised tomato internodes offer a mass flow system under physiological conditions (23). Perfusion experiments carried out with them and presented in this paper can answer the question of whether mass flow in plant vessels is compatible with different mass transfer rates of their contents. They might also reveal metabolic involvement of the xylem parenchyma cells in the longitudinal transfer of materials in the xylem vessels (13, 14).

MATERIALS AND METHODS

Plant Materials. Tomato plants (*Lycopersicon esculentum* cv. Moneymaker) were grown in Hoagland water cultures under a 25 C day and 18 C night. When they were about 1 meter high, the top was removed and "thefts" emerged out of the leaf axils. Fully developed first internodes of the thefts (mostly from 10- to 12-week-old plants) were cut off under water for experimental use.

Preparation for Perfusion. After saturation in 20 C tap water during 1 hr, a well closing silicone tube was carefully fitted around the morphological basal end of an internode. Then, it was taken out of the water, a plastic sheet was immediately wrapped around it to prevent transpiration, and a shorter silicone tube was fitted around the morphological apical end. Next, the internode was fixed, the morphological basal end on top, in such a position that the desired perfusion velocity (generally 1000 $mm^3\ hr^{-1}$) was reached. In this way, flow rate variations as a consequence of variations in xylem vessel number and their diameters can be corrected.

Perfusion of Radioactive Solution and Counting Procedure. Initially, 0.5 ml distilled H_2O was allowed to perfuse through the internode by gravity followed by 1 ml labeled solution, composed of 5 mM ^{14}C-sugars in tritiated H_2O or 5 mM ^{14}C- and ^{3}H-sugars in distilled H_2O. In 1 ml 1 μCi ^{14}C and 10 μCi ^{3}H were present as label. When the radioactive solution had passed the internode, 0.5 ml distilled H_2O was put into the upper silicone tube. After application of the radioactive solution, perfused drops of 50 μl were collected with microcaps every 3 min and were counted in 10 ml scintillate (4.9 g PPO, 0.1 g POPOP, 750 ml toluene, 250 ml methanol/l) in a liquid scintillation spectrometer.

After perfusion, the internode was freshly cut into pieces of 10 mm, which were ground separately in 0.2 ml 70% ethanol. From each fraction, an aliquot of 50 μl was counted in scintillation liquid.

The double label counting results were computed and plotted by the FORTRAN IV program SIPLOT.[1] The radioactive materials were supplied by the Radiochemical Centre, Amersham, U K. Specific radioactivities of $^{3}H_2O$, ^{14}C-D-sucrose, ^{14}C-D-glucose, ^{14}C-D-fructose, ^{3}H-D-sucrose, and ^{3}H-D-glucose were, respectively, 5 Ci/ml, 10 mCi/mmol, 3 mCi/mmol, 2.9 mCi/mmol, 2 Ci/mmol, and 2.3 Ci/mmol.

Some Theoretical Considerations. If a xylem vessel through which a solution is passing at a constant rate is regarded as a pipe with a wall permeable to solute and solvent, then a linear relationship between the logarithm of the concentration of the solute and the distance of movement from the point of entry into the stem may be expected. The slope of the curve will be directly related to the degree of leakage, regardless of the mechanism of escape. Mathematical treatments of these aspects of translocation have been given by Horwitz (11). The escape of the solute molecules may be of a physical kind, *viz.* diffusion out of the moving solution into the free space around it or exchange with charged constituents along the translocation path. It may also have a more metabolic character, for example, active absorption from the free space into the metabolic compartment of adjacent cells. Logarithmic removal of solutes from a vessel (11) is expressed as

$$C_p = C_o^{-KL/ApV} \quad (1)$$

or

$$K = 2.303 \log (C_o/C_p)/L \times ApV \quad (2)$$

[1] Available on request.

In the present experiments, K ($mm^2\ hr^{-1}$) is a constant, characteristic for the rate of escape of a compound from the vessel; C_o is the radioactivity applied at the top of the internode; C_p the radioactivity collected at the lower end; L (mm) is the length of the internode; Ap (mm^2) is the transverse-sectional area of the xylem vessels; V ($mm\ hr^{-1}$) is the velocity of flow.

In experiments with differently labeled compounds (*e.g.* ^{14}C-sucrose and ^{3}H-glucose) simultaneously perfusing through the same internode, the ratio of two K values can be expressed as

$$R = \log (C_o/C_p) \text{ sucrose}/\log (C_o/C_p) \text{ glucose} \quad (3).$$

RESULTS AND DISCUSSION

Preliminary perfusion experiments with acid fuchsin confirmed the finding (23) that the xylem vessels provide the principal translocation path in the present experiments.

All experiments showed similar leakage profiles of the labeled sugars. Three stages could be distinguished (Fig. 1) as discussed earlier for amino acids (22).

I. Filling of xylem vessels and xylem vessels walls, the free space of the system, until a steady state delivery was reached.

II. Steady state delivery during which a constant fraction of the perfusing, radioactive component was absorbed by the living cells around the xylem vessels. Absorption had already started in stage I and continued in stage III.

III. Washing out of dissolved compounds left in the xylem vessels and the neighboring free space.

^{14}C-sucrose was translocated much more rapidly than tritiated H_2O through the internode (Fig. 1). After sectioning the internode into pieces of 10 mm and measuring their radioactive content, the different longitudinal mass transfer rates appeared to be due to different escape constants for solute and solvent (Fig. 2). The internode showed longitudinal distribution profiles similar to those of phloem of intact plants after simultaneous transport of ^{14}C-sucrose and tritiated H_2O (5, 20). The ratio of lateral escape of $^{3}H_2O/^{14}C$-sucrose was about 6 (computed from the slopes of the lines in Fig. 2).

Lateral escape of H_2O in tomato internodes has been reported to be dependent on diffusion (21). Bull *et al.* (3) have reported a rapid exchange of tritiated H_2O from xylem vessels of sugarcane stalks into the storage tissue, so that its net longitudinal movement was low. They calculated that the entire water content of a stalk could be replaced under conditions of transpiration at least once a day. The present results suggest that a diffusional loss of tritiated H_2O alone can give rise to high escape constants (Figs. 1-3). Only 0.01% (Fig. 1A) and 0.09% (Fig. 1B) of the applied tritiated H_2O was recovered, which means that about 15% of the internodal H_2O content was replaced during the experiment. Internodal water content (6.7 ml) was determined from the loss of H_2O after freeze-drying tomato internodes of the same weight and length. In the experiment with successively perfusing sugars (Fig. 3) during which tritiated H_2O was administered twice, the tritiated H_2O recovery was 3.5% of the applied tritiated H_2O. As xylem vessels have at the most a volume of 100 mm^3 in the present experiments (*cf.* 8), the $^{3}H_2O$ must be absorbed in cells and cell walls around the xylem vessels.

The number and size of the xylem vessels, factors which determine perfusion velocity and absorption surface, have an effect on the rate of steady state delivery (Fig. 1) as was also found for amino acids (22). Furthermore, metabolic activities in the cells that surround the xylem vessels have been reported to be responsible for discrimination of the uptake of amino acids: L-alanine was taken up eight times as fast as α-aminoisobutyric acid in tomato internodes (22). The charge of the amino acids might play a role in their lateral escape through the negatively charged xylem vessel walls. Uptake of sugars is not supposed to be influenced by polar groups in the secondary cell walls, so that

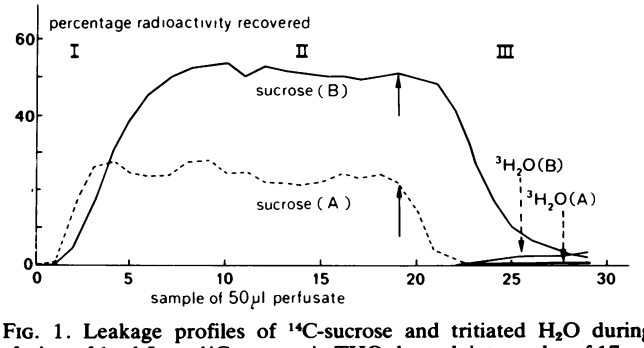

FIG. 1. Leakage profiles of ^{14}C-sucrose and tritiated H_2O during perfusion of 1 ml 5 mM ^{14}C-sucrose in THO through internodes of 17 cm under different flow rates (A, —, 640 $mm^3\ hr^{-1}$; B, ——, 1480 $mm^3\ hr^{-1}$). ↑, addition of 0.5 ml distilled H_2O. The percentage recovered represents the percentage of radioactivity collected per sample/radioactivity applied in 50 µl. For the interpretation of stages I, II, and III, see text.

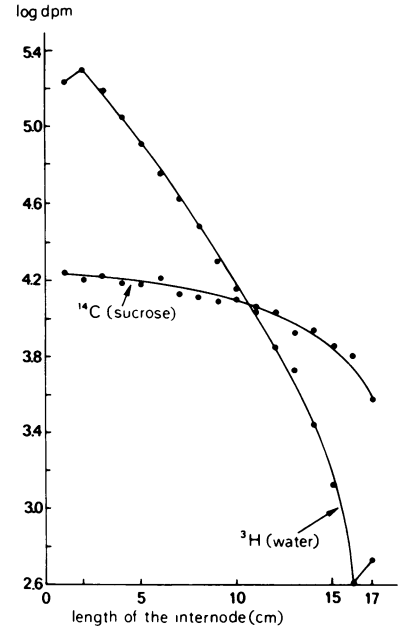

FIG. 2. Logarithmic distribution profile of radioactivity along an internode. Its perfusion profile is Fig. 1A.

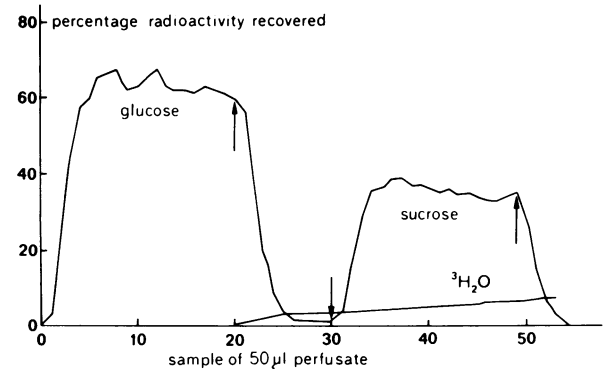

FIG. 3. Successive perfusion of 1 ml 5 mM ^{14}C-glucose in tritiated H_2O, 0.5 ml distilled H_2O (↑), 1 ml 5 mM ^{14}C-sucrose in tritiated H_2O (↓), and 0.2 ml distilled H_2O (↑). The percentage recovered represents the percentage of radioactivity collected per sample/radioactivity administered in 50 µl.

a discrimination of uptake can be thought to be mainly under metabolic control.

Discrimination of sugar uptake was tested by a successive perfusion procedure that should eliminate physiological and anatomical variations. The leakage pattern of glucose showed a higher steady state delivery than sucrose. A sucrose to glucose uptake ratio of 2.4 (Fig. 3) was computed from log (C_o/C_p) sucrose/log (C_o/C_p) glucose. This suggests metabolically controlled uptake, as diffusional loss from the xylem vessels would show a sucrose to glucose uptake ratio of 0.81 (computed from diffusion constants in water).

The preference of the surrounding parenchyma cells for sucrose over glucose was confirmed by experiments in which two sugars, one ^{14}C-labeled, the other ^3H-labeled, perfused simultaneously (Table I). Their leakage profiles were identical to those obtained by single perfusion. In all simultaneous two-sugar perfusion experiments, the sugars always exhibited the normal three-phasic delivery pattern. Sucrose was taken up 2.4 times as fast as glucose, which in turn was taken up 1.6 times as fast as fructose (Table I).

Different escape constants whatever the driving force may be, apparently cause different longitudinal mass transfer rates of the individual components in xylem vessels. Therefore, mass flow in sieve tubes has to be considered not in conflict with individual displacement of their contents (1, 5, 7, 9, 20).

An uptake ratio of sucrose to glucose greater than 0.81 suggests that tomato internode cells are capable of absorbing sucrose intact without intervention of the hydrolyzing enzyme invertase. This mechanism has been found to be functioning in sucrose uptake in other dicotyledons (12, 17, 18). Quite recently, it also has been found in tomato roots (6). In Table I, uptake ratios for sugars in tomato internodes have been compared with the uptake ratios into tobacco leaf discs (17), into immature internodal tissue of sugarcane (2), and into *Nitella flexilis* cells (24). It strengthens the assumption of Göring (10), that the capability to absorb sucrose intact is proper to dicotyledons.

The sucrose to glucose uptake ratio of *Saccharum* resembles that of *Nitella* (24). Sucrose must be hydrolzyed, before entering the cell. In that case, a competitive uptake has been found between sucrose and glucose (2, 24). The identical sucrose to glucose uptake ratios (2.4) during simultaneous (Table I) and successive (Fig. 3) perfusion experiments suggest no competition in their uptake and metabolic control by separate systems in tomato internodes.

In tomato internodes, the Q_{10} value for sucrose uptake was 3.5 (Fig. 4). Although a Q_{10} as high as 2.4 is reported for a diffusion-dependent sugar uptake in onion epidermis (19), it is assumed that the uptake of sucrose in tomato internodes is metabolically dependent, as generally a Q_{10} value higher than 2 is considered sufficient to regard uptake as such (24). In tobacco leaf veins too, sucrose uptake is determined to be metabolically dependent (4).

Table I. *Uptake Ratios (+SD) of Sucrose, Fructose, and Glucose in Some Plant Tissues*

From simultaneous perfusion experiments with ^3H- and ^{14}C-labeled sugars, the uptake ratios in *Lycopersicon* were computed by dividing their K values (see under "Some Theoretical Considerations," formula 3). The data are the average of five experiments. Ratios in other plants were adopted or computed from (2, 17, 24).

	Uptake ratio		
	sucrose/glucose	fructose/glucose	sucrose/fructose
Lycopersicon esculentum (present work)	2.43 ± 0.19	0.61 ± 0.10	3.52 ± 0.61
Nicotiana tabacum (17)		0.16	
Saccharum officinarum (2)	0.18	1.37	0.46
Nitella flexilis (24)	0.44	0.68	0.65

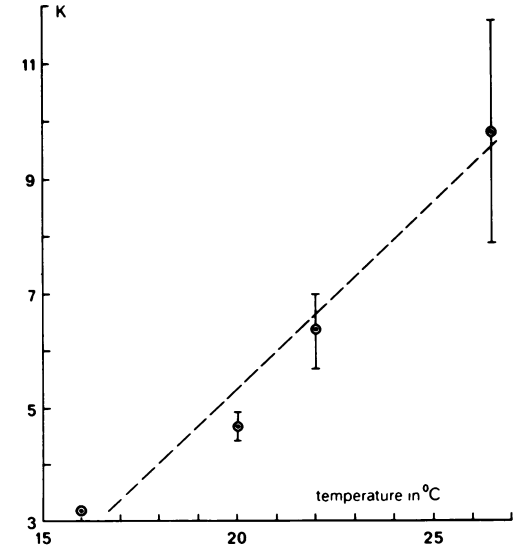

FIG. 4. Q_{10} Value for sucrose uptake in tomato internodes between 16 and 26 C. The uptake is expressed as K, the lateral escape constant (see under "Some Theoretical Considerations"), since it is assumed that the number of moles escaped is equal to that absorbed.

The results of this investigation indicate that the different longitudinal transfer rates of sugars in these gravity perfusion experiments through the xylem vessels of tomato internodes are dependent on metabolically controlled uptake by the cells surrounding the xylem vessels, most probably the xylem parenchyma cells (13, 14).

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