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Vein Loading: The Role of the Symplast in Intercellular Transport of Carbohydrate between the Mesophyll and Minor Veins of Tobacco Leaves

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ABSTRACT

Enzymatically separated leaf tissues of *Nicotiana tabacum* L., exhibiting good metabolic integrity, were used to evaluate the kinetics of sugar accumulation over the concentration range of 10 to 100 mM. Mesophyll cells exhibited Km values of 16 and 30 mM for glucose and sucrose, respectively; minor veins showed a reverse relationship, with Km values of 58 and 16 mM for glucose and sucrose, respectively. This would suggest that sucrose is preferentially absorbed by the minor vein net. Analysis of V_{max} data indicates a reduction in the ability of isolated minor veins to accumulate substrate, implicating a symplastic rather than apoplastic route for intercellular transport. Competition studies demonstrate a common carrier for sucrose and glucose in both tissue types and suggest the presence of a "transport compartment," entry to which is regulated by a critical intracellular sucrose concentration.

Photosynthate produced by fully expanded leaves is the primary source of sucrose for long distance transport. Although the processes by which sucrose is mobilized in the lamina of the leaf and accumulated in the phloem of the minor veins prior to transport are not well understood, they are crucially important in over-all plant growth.

Metabolic conversions of carbohydrates, as they exit the sieve element and are accumulated by metabolic sinks, have been extensively investigated. Through the use of sink tissues such as bean endocarp (22), immature storage tissues of sugarcane (3, 4, 5, 23), and cell suspensions of sugarcane parenchyma (20), models have been proposed for accumulation of carbohydrate by storage tissues. These models suggest a movement of carbohydrate through successive compartments, with invertase playing a critical role in the passage of carbohydrate from one compartment to another and in its subsequent immobilization in the storage compartment.

The problem of intercellular transport and subsequent loading of carbohydrate into the phloem of minor veins within the source leaf has been confounded by an inability to spatially separate carbohydrate-producing cells (mesophyll) from the loading or accumulating cells of the minor veins. Attempts to study the uptake or movement of sugars in heterogeneous leaf disks or primary vein segments have led to conflicting data (10, 16). Attempts to determine the mobile carbohydrate. (*i.e.*, sucrose or hexose) and its uptake kinetics have been complicated by the necessity to study uptake phenomena in leaf samples containing not only minor veins, but extensive amounts of photosynthetic mesophyll tissue. Such studies have yielded little information on the kinetics of uptake, because each component tissue possesses its own distinct affinity for substrate and potential for accumulation.

Although the minor vein net is extensive within the lamina of a dicotyledonous leaf, diffusion alone cannot account for the rapid export of photosynthate from leaves (16, 29). The movement of carbohydrate from photosynthetically active mesophyll cells to sieve elements can be via one of two possible pathways. The first consists of an apoplastic pathway for movement of carbohydrate from mesophyll cells to the phloem of minor veins. This involves expenditure of energy in crossing the plasmalemma of both mesophyll cells and the phloem cells involved in accumulation of carbohydrate prior to transport. The second involves a wholly symplastic pathway via plasmodesmata. Tyree (26) has studied the movement of solute molecules over short distances in various tissues and concluded that plasmodesmata were of sufficient frequency and diameter to enable the symplastic movement of solutes from cell to cell to be the pathway of least resistance. However, the possibility exists that both the apoplast and symplast may be involved. Sovonick *et al.* (25) suggested that, prior to vein loading, sugars move via the symplast within the mesophyll, enter the apoplast at the mesophyll-phloem parenchyma interface, and are subsequently transferred to the symplast of phloem cell types where they are actively accumulated.

The ubiquitous presence of plasmodesmata in plant tissues has prompted many workers to assign their function to intercellular communications and symplastic transport (21). There are two basic forms of plasmodesmata, examples of which are readily found in higher plant leaves. The common walls between mesophyll cells generally contain a simple form which is unbranched, whereas plasmodesmata between adjacent walls of phloem parenchyma, companion cells, and sieve tubes possess a much more complex, branched form. These branched forms in the minor veins have been implicated as possible sites of active transport prior to loading of carbohydrate into the sieve elements (13, 24). The precise role of the symplastic system in movement of carbohydrates between mesophyll and minor vein tissues is not yet clear.

The importance of understanding processes involved in movement of carbohydrate from areas of production to regions of utilization and storage is paramount if we ever expect to control the movement of photosynthate and remobilized

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materials into plant organs of economic value. In the present study, enzymatically isolated leaf tissues were used to resolve uptake kinetics of mesophyll and minor vein tissues separately, in an effort to elucidate the problem of intercellular transport and vein loading.

MATERIALS AND METHODS

Plant Material. *Nicotiana tabacum* L., 'Wisconsin 38,' was grown under greenhouse conditions and maintained as previously described (9). Uniform leaf material was obtained by selective newly expanded leaves from 8- to 10-week-old plants.

Leaves of similar developmental age were selected from the same plant at mid-morning and thoroughly washed in tap water before use. The lower epidermis was stripped from the interveinal lamina to enable rapid penetration of the digestive enzyme into the leaf disks. This enabled a comparison of short-term uptake kinetics for leaf disks with isolated mesophyll and vascular tissues, by minimizing the diffusional barrier presented by the lower epidermis to penetration of carbohydrate into the intact disks. Leaf disks of 0.6 cm^2 were sampled from the lamina, avoiding any large veins, and placed in an incubation medium containing 0.6 M sorbitol, 20 mM K_2SO_4, 1 mM KNO_3, 0.2 mM KH_2PO_4, 0.2 mM $MgSO_4$, 1 mM $CaCl_2$, 1 μM KI, 0.01 μM $CuSO_4$, and 5 mM $KHCO_3$.

Isolated tissues were obtained using procedures previously described (9). Young fully expanded leaves were washed, the lower epidermis was removed from the lamina between the primary leaf veins, and 0.6 cm^2 disks were excised. Tissue (1.0–1.5 g) was placed in 20 ml of the maceration medium (incubation medium containing 1% Macerozyme, Calbiochemical Inc.), vacuum infiltrated, placed in fresh maceration medium, and transferred to a reciprocating shaker bath (approximately 30 excursions per min) for 1.5 to 3.0 hr at 25 C and 10,800 lux of illumination. The mixture was next gently swirled for 30 sec on a "Vortex Genie" (Scientific Products) to separate the mesophyll cells from the minor vein net. The resultant suspension was filtered through a 40-μm nylon mesh, and the filtrate containing single mesophyll cells was centrifuged at 50 to 80*g* for 2 min to collect whole mesophyll cells. The minor vein bundles retained by the nylon mesh were washed and sorted, with only those bundles not containing mesophyll cells still attached being collected. Isolated tissues were resuspended in an incubation medium as per leaf disks.

Carbohydrate Uptake. Accumulation of ^{14}C-labeled sucrose, glucose, and fructose was followed over several concentration ranges (1–50, 10–150, and 100–500 mM) in the above incubation medium (containing 0.6 M sorbitol) at 25 C and 16,200 lux, with sorbitol levels being adjusted to compensate for the osmotic effect of the sugar under study. Disks and isolated tissues were allowed to accumulate sugars for various periods of time, and sugars were removed from the free space by leaching tissues for 4-min intervals in three changes of incubation medium. These three successive washes resulted in the removal of approximately 95% of the "free space" sugar. Tissues incubated or washed in solutions containing less than 0.05 M osmoticum appeared to be leaky. However, the addition of 1 mM $CaCl_2$ to the medium resulted in minimal leakage of sugars.

Uptake Kinetics. Kinetics of accumulation were followed with respect to time and substrate concentration. Determinations of the substrate concentration required to give half-maximal velocity (Km) and the maximum velocity at saturating substrate concentration (V_{max}) were made using Lineweaver-Burk plots (19).

Quantitative Analysis of Carbohydrates. The use of ^{14}C-sucrose, -glucose, and -fructose of known specific radioactivities (0.103, 0.10, and 0.12 μmole/μc, respectively) allowed a rapid conversion of dpm accumulated to μmole values. Uptake rates were routinely calculated as μmoles dm^{-2} hr^{-1} based on radioactivity in whole leaf disks and in disks of vascular tissues alone (leaf disks with mesophyll cells removed), each having an area of 0.6 cm^2, and in isolated mesophyll cells. The relative leaf area represented by suspensions of isolated mesophyll cells was estimated on a Chl basis from differences in Chl content between whole disks of laminae and isolated disks of bundles. Chlorophylls *a* and *b* were determined according to Arnon (2).

Radioactivity was determined using a Packard 2420 liquid scintillation spectrometer, using a scintillation mixture containing dioxane, 10% naphthalene, 0.7% PPO, and 0.03% POPOP (w/v). All counts were corrected for background and quenching.

RESULTS AND DISCUSSION

Parameters of Sugar Accumulation Using Leaf Disks. The effect of varying osmotic concentration of bathing media on uptake and subsequent leakage of sucrose was evaluated. The rate of sucrose uptake from graded sorbitol solutions containing 50 mM ^{14}C-sucrose was maximal with a bathing medium containing 0.05 M sorbitol. Increasing the osmoticum to 0.6 M inhibited uptake by 14%, whereas incubation on water containing 50 mM sucrose inhibited uptake by 33%. Removal of label from the free space was nearly complete after 10 min of washing in the presence of osmoticum, while leaching from the symplast continued after 20 min in disks incubated on water. This would be expected when tissues with an over-all negative water potential are placed in water alone, resulting in an increase in pressure potential (turgor) and a possible disruption of the plasmalemma.

Much of the controversy concerning an apoplastic *versus* symplastic pathway for movement of carbohydrate from mesophyll cells to the phloem revolves around the presence of an invertase in the cell walls of both sink tissues (23) and leaf tissues (7, 8). Although the presence of invertase in the "outer space" or cell wall is not refuted, its role in vein loading, as implicated by efflux experiments, is questioned. Kursanov and Brovchenko (18) demonstrated that 20 to 30% of the sugars accumulated by sugar beet mesophyll tissue can be washed from leaf tissues during a 30 min wash period. The leached sugars were in ratios similar to those found in the tissue. It is possible that these sugars, rather than representing an intercellular transport pool, may represent an abnormal leaching of sugars from the symplast as a result of excessive osmotic shock and membrane rupture resulting from incubation of small leaf pieces in water.

Mesophyll cells and vascular bundle isolates present a special problem in that they are osmotically fragile. In 0.3 M sorbitol, they exhibited a tendency to rupture and leak their cellular contents. For this reason they were maintained in an incubation medium containing 0.6 M osmoticum for all uptake studies. Isolates incubated in 50 mM ^{14}C-sucrose for 1 hr and placed in 0.6 M sorbitol medium showed the same reduced level of leakage from the symplast as seen with disks (unpublished data).

Preliminary studies evaluated the characteristics of sugar accumulation in leaf disks from solutions of sucrose, glucose, or fructose between 1 and 50 mM. Triphasic uptake curves were obtained for each of the sugars (Fig. 1, a and b). Sucrose exhibited apparent saturation of the carrier at concentrations of 25, 100, and 400 mM. Both glucose and fructose showed

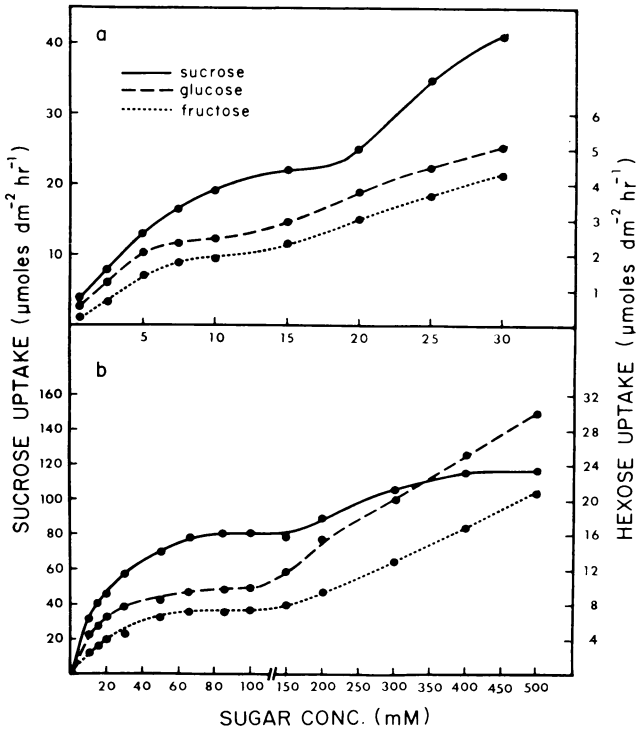

FIG. 1. Sugar accumulation curves for leaf disks incubated 1 hr on ^{14}C-sucrose, -glucose, or -fructose. Rates determined over the concentration range 1 to 30 mM (a), and 10 to 500 mM (b). Osmotic concentration of sugar and sorbitol in bathing media was adjusted to 0.6 M with sorbitol. Duplicate samples of three disks, each 0.6 cm^2, were used for each treatment and the counts averaged.

saturation at 8 and 100 mM. There was a linear increase in uptake of glucose and fructose which does not conform to Michaelis-Menten kinetics at substrate concentrations above 150 mM. A biphasic uptake curve has been described for sucrose uptake by cotyledons of excised bean seedlings (14, 15). However, the cotyledons did not show linear uptake above 150 mM.

Linear uptake (constant slope) of ^{14}C-labeled sucrose, glucose, and fructose from 30 mM solutions was observed for 15 hr for leaf disks. Intracellular concentrations of these sugars in leaf disks prior to uptake studies were calculated, based on enzymatic analysis procedures and estimates made of pool distribution within the cell. Assuming that sugars were uniformly distributed throughout the cell, internal concentrations of sucrose, glucose, and fructose were 120, 30, and 50 mM, respectively. Thus, accumulation from a 30 mM solution for 15 hr would necessitate uptake against a concentration gradient and the expenditure of metabolic energy. Addition of 1 mM KCN, a metabolic inhibitor, resulted in an immediate cessation of uptake, suggesting an active, energy-dependent process. Sugar uptake by isolated mesophyll cells and vascular bundles resulted not only in a cessation of accumulation but also a slight leakage of the labeled sugar in the presence of 1 mM KCN (unpublished data).

Kinetics of Sugar Uptake by Leaf Disks and Their Isolated Tissues. Previous studies have either disregarded the specificity of individual tissues and considered the leaf as a functional organ, or else, arbitrarily defined interveinal lamina (mesophyll and minor veins) as mesophyll, and dissected bundles (level 3 and 4 vascular bundles [11]) as having functions comparable with the minor vein net. Lack of techniques that yielded homogeneous systems of mesophyll cells and minor veins previously necessitated use of these indirect approaches to study kinetics of sugar accumulation.

The ability to physically study sugar uptake by minor veins devoid of adjacent mesophyll cells should provide an excellent system to study symplastic *versus* apoplastic uptake. If symplastic uptake predominates, uptake by isolated minor veins should be low when compared with that of intact disks, while it should be equal to or greater than that of intact disks if apoplastic uptake is the preferred pathway. This is based on the assumption that the disks of isolated veins are intact except for the disruption of plasmodesmata between border parenchyma cells and adjacent mesophyll cells, which are critical in symplastic transport and less significant in apoplastic transport occurring via the common cell walls.

Kinetic parameters (Km, V_{max}) were calculated from double reciprocal plots, to evaluate the ability of leaf disks and isolated tissues to accumulate exogenously supplied carbohydrate. Preliminary studies showed efflux of both labeled and unlabeled sugars to be negligible compared to influx of the labeled species over a 4-hr period (unpublished data). The magnitude of V_{max} is then dependent on the number of carrier sites. Assuming that the number of active carrier sites remains constant for any experimental treatment, then changes in V_{max} with time reflect the ability of the carrier to release and compartmentalize the released substrate in such a way as to minimize the inhibition of uptake caused by product accumulation. Analysis of sugar accumulation rates by both disks and isolated tissues showed uptake to be linear for at least 1 hr. Subsequently, 1-hr uptake periods were employed in all kinetic experiments to evaluate the ability of leaf tissues to handle physiological quantities of carbohydrate, since initial uptake kinetics would not serve this purpose.

Preliminary experiments using 10 to 100 mM ^{14}C-carbohydrate indicated that the affinity (Km) of disks and tissues for glucose and sucrose varied substantially with physiological age of leaf tissue and made kinetic analysis difficult, because linear relationships could not be obtained using Lineweaver-Burk plots. Leaves at 50% expansion, the physiological age at which they first become exporters of photosynthate, exhibited a high affinity for exogenously supplied sugars (Km = 3–8 and 10–20 mM for glucose and sucrose, respectively). At full expansion, the apparent affinity of disks for sugars is reduced to 15 to 25 and 30 to 40 mM for glucose and sucrose, respectively. The Km obtained for sucrose was comparable to that obtained for bean leaf tissue by Vickery and Mercer (27, 28). Disks and their isolates were taken from the same leaf for each experiment to eliminate problems of variability. A newly, fully expanded leaf was chosen in each case.

A comparative analysis of Km and V_{max} for glucose and sucrose uptake by disks, mesophyll cells, and minor veins revealed that Km values for disks and isolated mesophyll cells were similar, with both showing a higher affinity for glucose than sucrose. Isolated minor veins had a higher affinity for sucrose (Table I). This difference suggests that previous studies comparing leaf segments and larger leaf veins may not have evaluated the loading process *per se*, but compared accumulation patterns of mesophyll *versus* transport tissues not directly associated with the loading process. Kursanov *et al.* (17) and Brovchenko (6, 7) have consistently shown that vascular traces (not minor veins) dissected from the lamina of sugar beet accumulated glucose to a greater extent than sucrose; yet the minor veins of tobacco enzymatically isolated in these experiments showed a greater affinity for sucrose (Km = 16.1 mM). Assuming that tobacco and sugar beet leaves possess a similar loading system, it is plausible that the uptake patterns obtained by the above authors may have resulted from accumulation by lateral storage cells (sinks) present in the larger veins, and did

Table I. *Kinetic Data for Sugar Uptake (10 to 150 mM) by Tobacco Leaf Disks and Separated Tissues*

Uptake period was 1 hr. The units are Km = mM; V_{max} = μmoles dm^{-2} hr^{-1}.

Tissues	Sucrose		Glucose	
	Km	Vmax	Km	Vmax
Disks	32.3	109.5	16.7	20.1
Mesophyll	31.3	9.1	16.7	3.9
Minor veins	16.1	11.8	58.8	5.3

not represent absorption by phloem tissues of the minor vein (11–13).

At saturating concentrations of glucose and fructose, V_{max} for sugar uptake by tobacco leaf disks was distinctly higher than for either of the isolated tissues (Table I). Based on the metabolic integrity of these isolates (9), it is doubtful that the plasmalemma (site of carrier-mediated transport) was damaged, aside from a severance of the intercellular plasmodesmata. With this assumption, and assuming a minor role for the plasmodesmata in sugar accumulation by the minor veins in apoplastic transport, isolated bundles should have had a V_{max} for exogenous sugar at least approximating the rates measured for disks. Since this was not the case, the data suggest a minor role for apoplastic movement of sugars from mesophyll cells to adjacent minor veins.

Specificity of the Transport Site. The observation that distinctly different Km values exist for glucose and sucrose in each of the isolated tissues leads to several possibilities concerning active transport of sugars in these tissues. Is it possible that the carriers for carbohydrates differ between mesophyll cells and minor veins, or are the differences in affinity the result of compartmentalization and substrate modification following release from the carrier site? Two approaches were selected in evaluating the carrier system in these two tissues. The first involved an analysis of the pattern of competition between glucose and sucrose for the carrier site; the second approach was to follow the uptake velocity (V_{max}) with time in an effort to assess whether there were obvious compartmentalization differences.

Disks, mesophyll cells, and minor veins were incubated in a graded series containing 10 to 100 mM ^{14}C-sucrose, with and without added glucose (Fig. 2). These plots are typical of competitive inhibition and suggest that glucose and sucrose compete for the same carrier site in the two tissue types. When these tissues were incubated in 10 to 100 mM ^{14}C-glucose, with and without added sucrose (Fig. 3), the presence of sucrose at concentrations below 5 mM gave the typical competitive inhibition plots. However, at concentrations above 6.6 mM (apparently saturating at 10 mM), the presence of sucrose in the incubation media resulted in a decrease in Km and an accelerated uptake of glucose. Although these results were consistently obtained for disks and bundles, mesophyll cells exhibited erratic results under similar conditions (unpublished data).

Assuming that compartmentalization of cellular metabolites is essential to operation of the metabolic machinery, these uptake patterns suggest a dynamic change in the access of cellular carbohydrates to one or more metabolic pools. Apparently, glucose, a metabolic intermediate essential for normal operation of cellular metabolism, and sucrose, a specialized compound not readily accessible to metabolic processes (1), must be spatially separated. Although a common carrier is utilized, intracellular concentrations of glucose and sucrose may be closely regulated with excess hexose being shunted (as sucrose) from normal metabolic compartments to a special transport compartment for export out of the leaf. This hypothesis is reinforced by data showing that glucose absorbed by leaf cells

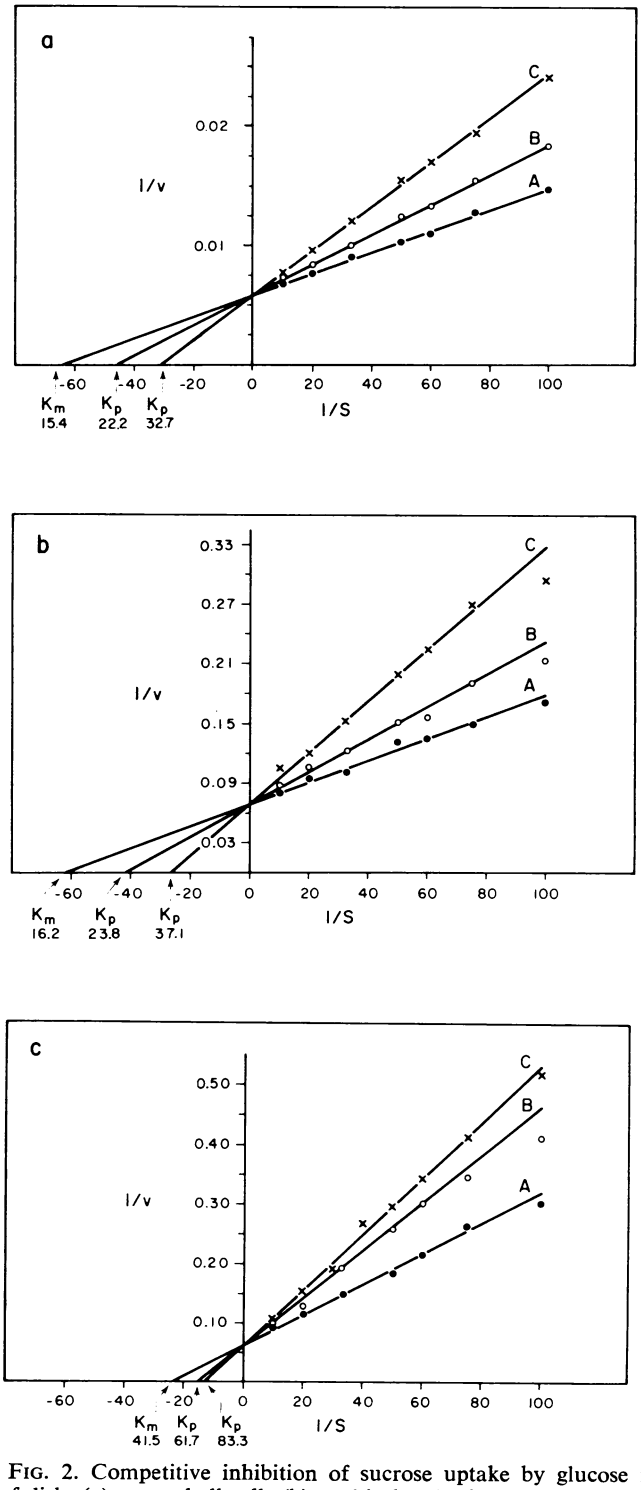

FIG. 2. Competitive inhibition of sucrose uptake by glucose in leaf disks (a), mesophyll cells (b), and isolated minor vein (c). Disks and isolated tissues were incubated in 10 to 100 mM sucrose alone (A), or in the presence of 6.6 or 20 mM glucose (B and C, respectively). Uptake velocity (v) = μmoles dm^{-2} hr^{-1}, and S = concentration (M) of sucrose in the medium. Km and K_p represent the Michaelis constants in the absence and presence of a competitive inhibitor, respectively.

is rapidly converted to sucrose, though the reverse is not true for absorbed sucrose (unpublished data). In the competition experiments using ^{14}C-glucose, the presence of 10 mM sucrose may increase the intracellular level of sucrose sufficiently to permit accumulated glucose to be diverted from the metabolic compartment to the transport compartment. The inability of isolated mesophyll cells to exhibit accelerated uptake of glucose in the presence of sucrose may have resulted from the absence of a sink (minor veins) and hence utilization of the transport compartment.

The kinetics of sugar uptake by leaf disks and isolated tissues were followed over a 4-hr period to investigate the theory of multiple compartments. Disks exhibited a high, relatively sustained V_{max} for sucrose uptake (Table II), whereas the velocity of glucose uptake at saturating substrate concentration increased over the 4-hr period and is consistent with the theory of multiple compartments. Isolated mesophyll cells and minor veins showed a decrease with time in the V_{max} for both glucose and sucrose uptake. The inability of these isolated tissues to maintain sucrose uptake and to exhibit an increased time dependent uptake of glucose would result if the transport compartment, extending between producing cells (mesophyll) and transport cells (minor veins), is relatively small.

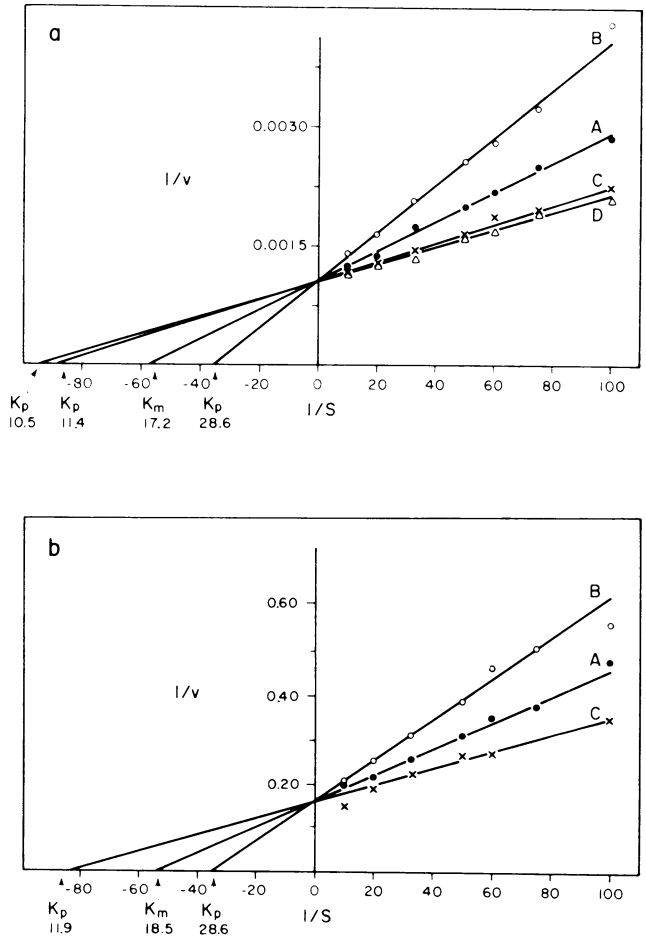

FIG. 3. Competitive inhibition of glucose uptake by sucrose in leaf disks (a) and isolated minor veins (b). Disks and isolated minor veins were incubated in 10 to 100 mM glucose alone (A), and in the presence of 1.3, 6.6, or 50 mM sucrose (B, C, and D, respectively). Uptake velocity (v) = μmoles dm^{-2} hr^{-1}, and S = concentration (M) of glucose in the medium. *Km* and K_p represent the Michaelis constants in the absence and presence of a competitive inhibitor, respectively.

Table II. *Changes in V_{max} Associated with Time of Accumulation by Disks and Isolated Tissues, Km and V_{max} Determined from Lineweaver-Burk Plots*

Tissue	Labeled Sugar Present	K_m	Duration of Accumulation			
			1 hr	2 hr	3 hr	4 hr
		mM	V_{max} (μmoles dm^{-2} hr^{-1})			
Disks	Sucrose	33.7	121.1	120.9	110.6	99.6
	Glucose	19.1	10.2	11.9	20.4	26.8
Mesophyll	Sucrose	34.5	10.2	9.0	6.1	5.7
	Glucose	18.9	4.6	4.0	2.7	2.0
Minor veins	Sucrose	15.2	12.1	11.3	8.4	8.2
	Glucose	52.7	5.7	5.3	3.9	3.1

If vein loading occurs via a symplastic route, as the data suggests, then the role of both the plasmalemma and the plasmodesmata must be considered. Either of these membrane structures may contain the sites for carrier-mediated transport. A symplastic transport compartment would not only serve to isolate sucrose from metabolic utilization, but would provide a direct route for the rapid movement of carbohydrate from producing cells to the phloem. Isolated minor veins with their severed channels may be incapable of effectively accumulating sucrose into the transport compartment as can the intact disks. Under these conditions, glucose and sucrose may have access only to the metabolic compartment via the plasmalemma.

The use of enzymatically isolated leaf tissues has permitted a kinetic analysis of the processes involved in the intercellular transport of carbohydrate into and between the mesophyll cells and minor veins in leaves of tobacco. Leaf disks and isolated mesophyll cells exhibited a *Km* of 16 mM for glucose and 30 mM for sucrose. Isolated minor veins showed a reverse affinity with a *Km* of 58 and 16 mM for glucose and sucrose, respectively. With minor veins exhibiting a higher affinity for sucrose, it is difficult to rationalize a system where hexose would be the sugar absorbed by the minor veins prior to long-distance transport in the sieve tubes. Competition studies using glucose as a competitor with ^{14}C-sucrose uptake indicated a competitive type inhibition and, therefore, a common carrier for uptake of both sucrose and glucose. When sucrose was used as a competitive inhibitor with ^{14}C-glucose uptake, a decrease in *Km* for glucose (increased affinity of the carrier for substrate) was observed at sucrose concentrations above 6.6 mM. This increased affinity of the carrier suggests a modification in either the carrier or the cellular disposition of accumulated glucose. It is possible that an alternate carrier for glucose is present in the membrane and is activated by the presence of sucrose. The data can also be explained by postulating the transfer of excess glucose to a special metabolic compartment for subsequent transport to the minor veins when sucrose is in high concentration.

Analysis of the absolute velocity for accumulation at saturating substrate concentration (V_{max}) indicated that isolated mesophyll cells and minor veins no longer accumulated carbohydrate to the extent exhibited by leaf disks. This suggests that physical disruption of the symplast (plasmodesmata) limits the ability of minor veins to actively absorb sugar, and therefore supports the hypothesis that intercellular transport within the lamina occurs via a symplastic rather than apoplastic route. Subsequent analysis of V_{max} for glucose and sucrose over a 4-hr period using disks and isolated tissues support the presence of a hypothetical transport compartment (endoplasmic reticulum and plasmodesmata system) which requires a degree of symplastic integrity for its function.

The data support a symplastic pathway for movement of photosynthate from mesophyll to vascular tissues. It is proposed that sugars, following uptake or produced by photosynthesis, are taken into a transport compartment, consisting of the endoplasmic reticulum, rapidly moved via plasmodesmata to the border parenchyma where they are subsequently transported to companion cells and then deposited in sieve elements for long-distance transport. The form of the mobile species is most likely sucrose, but the data do not exclude the possibility of interconversions such as phosphorylation (*i.e.*, sucrose phosphate or hexose phosphate) along the transport pathway.

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