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Transport and Metabolism of 1′-Fluorosucrose, a Sucrose Analog Not Subject to Invertase Hydrolysis[1]

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ABSTRACT

The novel sucrose derivative 1′-fluorosucrose (α-D-glucopyranosyl-β-D-1-deoxy-1-fluorofructofuranoside) was synthesized in order to help define mechanisms of sucrose entry into plant cells. Replacement of the 1′-hydroxyl by fluorine very greatly reduces invertase hydrolysis of the derivative (hydrolysis at 10 millimolar 1′-fluorosucrose is less than 2% that of sucrose) but does not reduce recognition, binding, or transport of 1′-fluorosucrose by a sucrose carrier. Transport characteristics of 1′-fluorosucrose were studied in three different tissues. The derivative is transported by the sucrose carrier in the plasmalemma of developing soybean cotyledon protoplasts with a higher affinity than sucrose (K_m 1′-fluorosucrose 0.9 millimolar, K_m sucrose 2.0 millimolar). 1′-Fluorosucrose is a competitive inhibitor of sucrose uptake with an apparent K_i also of 0.9 millimolar, while the K_i of sucrose competition of 1′-fluorosucrose uptake was 2.0 millimolar. Thus, both sugars are recognized at the same binding site in the plasmalemma. Both sucrose and 1′-fluorosucrose show very similar patterns of phloem translocation from an abraded leaf surface through the petiole indicating that recognition of 1′-fluorosucrose by sucrose carriers involved in phloem loading is likely as well.

1′-Fluorosucrose is a very poor substrate for invertase and as such is absorbed only slowly by corn root segments, a tissue in which sucrose hydrolysis by a cell wall invertase is required prior to active hexose uptake.

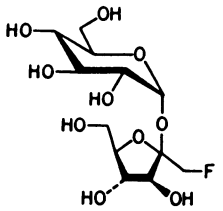
The kinetics of 1′-fluorosucrose uptake by soybean cotyledon protoplasts indicate that membrane passage and substrate release to the protoplast interior are rate limiting to transport. Recognition of sucrose at the inner membrane surface of the carrier protein is apparently different than recognition and binding at the external surface.

Sucrose is the major transportable product of photosynthesis in most plants, yet comparatively little is known about the details of its synthesis, metabolism, or transport at the cell membrane level. The study of sucrose transport has been limited, in part, by the lack of suitable nonmetabolizable analogs (*i.e.* the equivalent of 2-deoxy- or 3-*O*-methyl-glucose).

In general, the active transport of sucrose across cell membranes proceeds either through a sucrose-specific carrier or requires extracellular hydrolysis by an extracellular invertase prior to uptake of hexoses (5, 6). Resolution of these two mechanisms has relied on the degree of radiolabel randomization observed after uptake of asymmetrically labeled sucrose, on inferences made from the localization of sucrose-metabolizing enzymes, on transport of sugars into protoplasts from specific cell types, or a combination of these techniques (5). When several tissue types lie in the transport path, as is often the case, determination of the uptake mechanism can be quite difficult and laborious.

Extracellular invertase is primarily responsible for sucrose hydrolysis in the apoplast; therefore, a sucrose analog which is recognized by sucrose transporters, but which is not subject to hydrolysis by invertase, would be desirable for the study of sucrose transport in a variety of plant tissues. Since substrate recognition by invertase requires unsubstituted hydroxyl groups at the fructose C-1 and C-6 positions of fructosides (7) and substrate recognition by the sucrose carrier protein in soybean cotyledon protoplasts is quite specific for disaccharides structurally similar to sucrose (10), it seems likely that modification of the fructosyl portion of sucrose to reduce or eliminate invertase hydrolysis must be minimal and carefully chosen in order to retain recognition by sucrose carrier proteins.

We have recently described the synthesis of one such substituted sucrose; 1′-fluorosucrose:



and in this report describe its transport and metabolic properties in plant tissues. We demonstrate that 1′-fluorosucrose is very poorly hydrolyzed by invertase, is transported by a sucrose carrier in soybean protoplasts, is translocated in the phloem, and is metabolized much more slowly than sucrose.

MATERIALS AND METHODS

General. 1′-Deoxy-1′-fluorosucrose was prepared as described previously (4). In brief, 1-deoxy-1-fluorofructose was prepared by displacement of the 1-trifluoromethanesulfonyl group of 1-trifluoromethanesulfonyl-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranoside using Tris(dimethylamino) sulfonium difluorotrimethylsilicate and subsequent hydrolysis of the isopropylidene groups to give the free 1-fluorofructose. The latter was coupled enzymically to give 1′-fluorosucrose using either [^{12}C]- or [^{14}C] (glucosyl-UDP-glucose and sucrose synthetase isolated from barley seeds. Growth of Wye soybeans, isolation of protoplasts from soybean cotyledons, and measurement of [^{14}C]sucrose or 1′-[^{14}C] fluorosucrose uptake rates were recently described (8).

Invertase Hydrolysis of Sucrose or 1′-Fluorosucrose. Glucose produced by the hydrolysis of sucrose or 1′-fluorosucrose by yeast invertase (Sigma) was assayed spectrophotometrically in a reaction solution containing 10 μmol ATP, 10 μmol NADP, 10 μmol $MgCl_2$, and approximately 1 unit each of hexokinase and

[1] Contribution No. 3504.

glucose-6-phosphate dehydrogenase in 0.5 ml of 25 mM citrate-Hepes-Mes buffer (pH 6.0).

The ability of plant invertases to hydrolyze 1′-fluorosucrose was evaluated by determining glucose production in nonfractionated extracts of soybean leaves. Mature "Wye" soybean leaves (midrib removed) were ground thoroughly in 2 volumes of ice-cold buffer (100 mM Hepes-KOH [pH 7.5], 5 mM DTT, 15 mM EDTA) in a prechilled mortar and pestle, and the mortar rinsed with a further 2 volumes of buffer. The extract and rinse were combined and centrifuged for 0.5 h at 40 k*g* and 4°C. The clear supernatant was passed through a PD-10 (Pharmacia) desalting column equilibrated with 5 mM Hepes-KOH (pH 7.5), 5 mM DTT, and 0.75 mM EDTA at room temperature. Aliquots of the eluate were assayed for invertase activity by incubation with the appropriate concentration of sucrose and/or 1′-fluorosucrose in 25 mM citrate-Mes-Hepes-KOH, 5 mM DTT (pH 5.0) for 1 h at 30°C. After the reaction was terminated by incubation in a 100°C bath for 5 min, glucose produced was assayed using the "Flozyme" reagent (Worthington Biochemicals). As a control, extracts were treated in the 100°C bath for 5 min immediately after addition to the substrate-containing reaction mix and the resulting glucose production subtracted from that observed in the experimental treatments.

[^{14}C]-1′-Fluorosucrose and [^{3}H]Sucrose Translocation. Trifoliolates at podbearing nodes of Wye soybeans at 52 to 60 d after planting were selected and detached. Trifoliolates selected had petioles 15 to 17 cm long. The two lateral leaflets were removed and two, 3.9 cm^2 circles, one at each leaf margin, were abraded with carborundum. The leaflet was placed in a covered container with the petiole extending into a receiving flask. The petiole was then recut under a solution of 20 mM EDTA, and placed into a receiving vial containing 1 ml of 20 mM EDTA to collect phloem exudate. Twenty μl of a solution 25 mM in each [^{14}C](glucosyl)-1′-fluorosucrose (0.5 μCi/μmol) and [^{3}H](fructosyl)sucrose (0.75 μCi/μmol) was applied to each abraded spot. The 20-mM EDTA receiving solution was removed for scintillation counting at 15-min intervals and replaced with 1 ml of fresh solution. At 1 h after addition of the radiolabeled sucrose and 1′-fluorosucrose solutions, the abraded areas were flooded with 50 μl of a solution 100 mM in both 1′-fluorosucrose and sucrose. Approximately 1 min was allowed for penetration and the solution was removed by aspiration. The washout procedure was repeated four times, with the final wash solution allowed to remain on the abraded spot.

Uptake and Metabolism of [^{14}C]-1′-Fluorosucrose or [^{14}C]Sucrose by Corn Root Tissue. Uptake of [^{14}C](glucosyl)-1′fluorosucrose or [^{14}C]sucrose into corn root segments was measured as described by Giaquinta *et al.* (6) except that the uptake solution was 1 mM in sugar at 1 μCi/μmol. Tissue extracted after 1 h incubation was used for determination of metabolite distribution.

Metabolism of [^{14}C]-1′-Fluorosucrose or [^{14}C]Sucrose by Soybean Leaf Discs. Leaf discs (1.2 cm diameter) were punched from the margins of the center leaflet of a fully expanded trifoliolate. Fifteen discs were vacuum infiltrated with 1 mM [^{14}C](glucosyl)-1′-fluorosucrose or 1 mM [^{14}C]sucrose in 0.2 mM $CaCl_2$, then incubated for 2 h at 22°C in room light. Samples of five discs each were washed four times with 0.2 mM $CaCl_2$ at 0°C before fractionation as described previously (6).

RESULTS

Invertase Catalyzed Hydrolysis. Figure 1 shows a comparison of the rates of 1′-fluorosucrose and sucrose hydrolysis by purified yeast invertase as a function of substrate concentration. Since the hydrolysis rate for 1′-fluorosucrose does not approach saturation at substrate concentrations well above those saturating sucrose hydrolysis, the primary effect of the 1′-fluoro substitution in reducing invertase activity against the substrate is apparently a large increase in K_m. From the available data, V_{max} for 1′-fluorosucrose cannot be reliably determined so that whether or not V_{max} has also been altered is not clear. 1′-Fluorosucrose hydrolysis by an extract from soybean leaves gave similar results in that hydrolysis of 5 mM sucrose proceeded at 10 times the rate of 50 mM 1′-fluorosucrose.

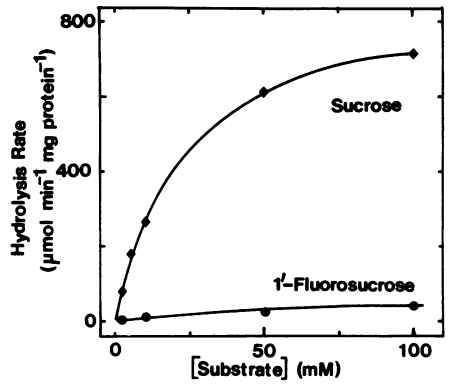


FIG. 1. Hydrolysis of sucrose or 1′-fluorosucrose by purified yeast invertase as a function of substrate concentration. The hydrolysis rate was measured by glucose appearance at pH 6.0 and 26°C in a coupled enzyme assay as described in "Materials and Methods".

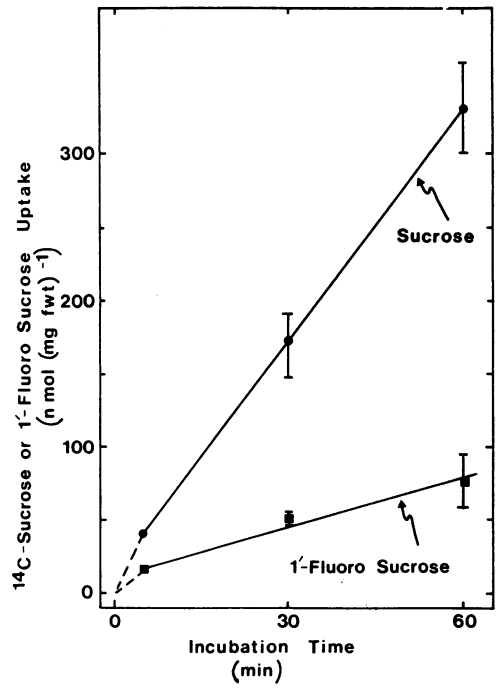


FIG. 2. The time course of the uptake of 1 mM [^{14}C]sucrose (●) or 1′-[^{14}C]fluorosucrose (○) by corn root segments. Brackets are ±SE of the mean for three assays.

Uptake of [^{14}C]-1′-Fluorosucrose by Corn Root Segments. Sucrose exogenously applied to corn root segments is accumulated within the cells only after extracellular hydrolysis by invertase (6). Incubation of corn root segments in either 1 mM [^{14}C]sucrose or 1 mM [^{14}C](glucosyl)-1′-fluorosucrose resulted in a linear uptake (Fig. 2); however, the rate of [^{14}C]sucrose uptake was about four times the rate of [^{14}C](glucosyl)-1′-fluorosucrose uptake. This result suggests that cell wall invertase is also incapable of 1′-fluorosucrose hydrolysis at these concentrations.

Uptake of [^{14}C]-1′-Fluorosucrose and [^{14}C]Sucrose by Soybean Cotyledon Protoplasts. We have recently shown that protoplasts

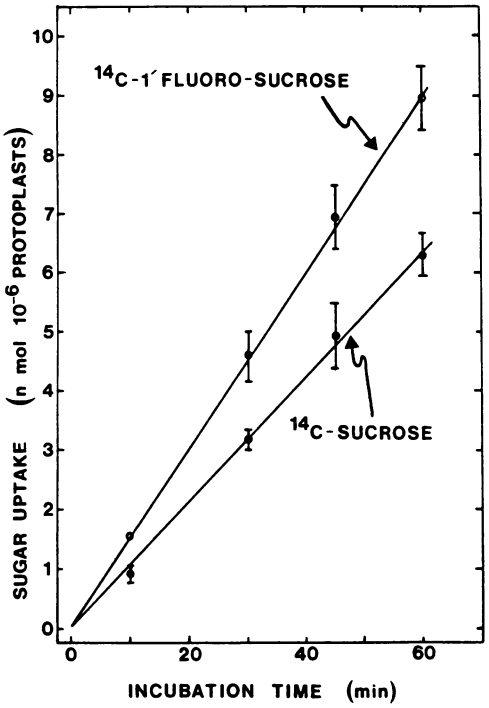


FIG. 3. The time course of the uptake of 0.5 mM [^{14}C]sucrose (●) or [^{14}C](glucosyl)-1'-fluorosucrose (■) by soybean cotyledon protoplasts. Brackets are ±SE of the mean of three assays. Specific radioactivities were 2.47 μCi/μmol for sucrose and 3.3 μCi/μmol for 1'-fluorosucrose.

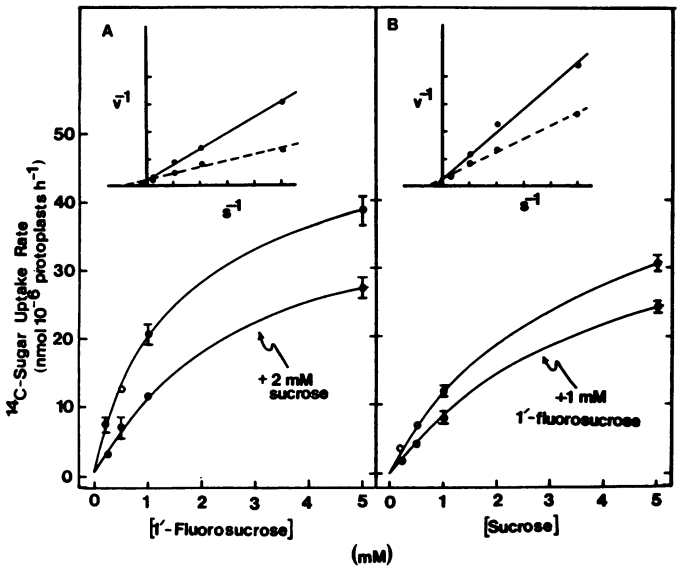


FIG. 4. A, Influx of [^{14}C](glucosyl)-1'-fluorosucrose *versus* concentration of 1'-fluorosucrose, with or without 2 mM sucrose; B, influx of [^{14}C] sucrose *versus* sucrose concentration with or without 1 mM 1'-fluorosucrose. Brackets are ±SE of the mean of four determinations. Insets, Double reciprocal plots of the primary data.

isolated from the cotyledons of developing soybeans accumulate sucrose through an energy-dependent, sucrose-specific carrier (8, 10) and have therefore used that system to characterize the uptake of 1'-fluorosucrose.

Uptake of either ^{14}C-sugar at 0.5 mM external sugar concentration was linear with time for at least 1 h (Fig. 3); however, the influx of [^{14}C](glucosyl)-1'-fluorosucrose was about 30% faster than the linear rate of [^{14}C]sucrose uptake at this low substrate concentration. Results of a typical experiment in which soybean

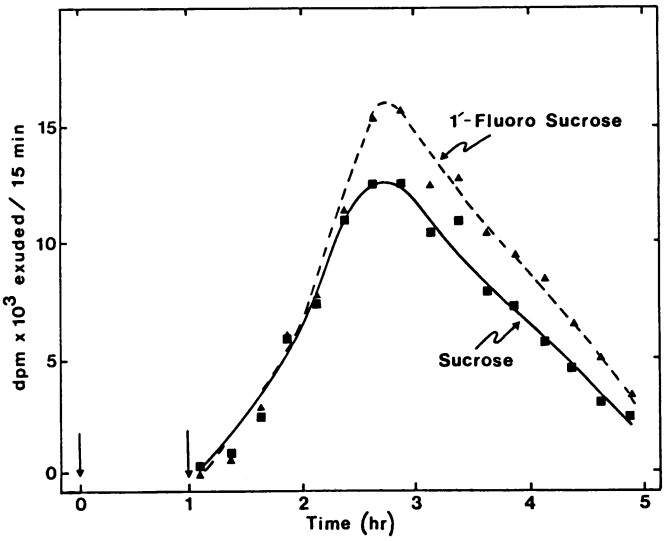


FIG. 5. The arrival of [^{3}H]sucrose and [^{14}C](glucosyl)-1'-fluorosucrose into a receiving solution of 20 mM EDTA at the cut end of the petiole, after application to abraded spots on the leaf surface. Radiolabeled solutions were applied at time 0 as indicated by the first arrow on the graph. The nonradiolabeled chase was added at the second arrow.

cotyledon protoplasts from a single isolation were incubated with varying concentrations of either [^{14}C](glucosyl)-1'-fluorosucrose, [^{14}C]sucrose,[^{14}C](glucosyl)-1'-fluorosucrose with added [^{12}C]sucrose, or [^{14}C]sucrose with added 1'-[^{12}C]fluorosucrose are shown in Figure 4. The resulting kinetic data were fit to the multiple-component model described previously (8, 10). The apparent K_m for sucrose uptake by the saturable component in typical protoplast preparations was in the range 1.5 to 2.5 mM (1.65 mM in the experiment shown in Fig. 4) as reported previously, while the K_m for 1'-fluorosucrose (0.87 mM) was approximately one-half the K_m for sucrose. The apparent V_{max} (38 nmol 10^{-6} protoplasts h^{-1}) of the saturable component was the same for both substrates.

Both [^{12}C]sucrose and [^{14}C]-1'-fluorosucrose, when added to ^{14}C-labeled uptake solutions of the opposite analog act as competitors for the carrier mediated influx of the labeled substrate (Fig. 4). In several experiments of this type, the K_m for uptake of one substrate was approximately equal to its K_i when used as a competitor for the alternate substrate. Both sugars apparently occupy the same site on the sucrose carrier protein; however, 1'-fluorosucrose binds with slightly higher affinity than the natural substrate.

Translocation of [^{14}C]-1'-Fluorosucrose in Mature Soybean Leaflets. When [^{14}C](glucosyl)-1'-fluorosucrose and [^{3}H](fructosyl)-sucrose were simultaneously supplied to abraded surfaces on mature soybean leaflets, the pattern of label efflux from the cut petiole into EDTA solution was the same for both compounds (Fig. 5).

The time from label application to appearance at the receiving solution, the timing and extent of label chase at the receiving flask, and the total label delivered, all varied from leaflet to leaflet; however, the two compounds consistently showed the same pattern within a leaflet. Thus, 1'-fluorosucrose is also recognized by sucrose transporters in source leaves and is translocated in the phloem.

Metabolism of [^{14}C]Sucrose and [^{14}C]-1'-Fluorosucrose. A large proportion of label from either exogenously supplied compound remained in the neutral solubles after extraction of leaf discs and corn root segments (Table I). In both tissues, however, about five times more label from sucrose appeared in combined acidic and basic solubles than was converted from [^{14}C](glucosyl)-1'-fluo-

Table I. *Metabolism of [^{14}C]-1′-Fluorosucrose and [^{14}C]Sucrose by Corn Root Segments or Soybean Leaf Discs*

	Soybean Leaf Discs[a]		Corn Root Segments[b]	
	[^{14}C]-1′-Fluorosucrose	[^{14}C]Sucrose	[^{14}C]-1′-Fluorosucrose	[^{14}C]Sucrose
	% of ^{14}C recovered[c]			
Total neutral solubles	90.2 ± 4.6	73.8 ± 0.1	92.5 ± 1.1	60.7 ± 3.2
Acidic + basic solubles	3.2 ± 0.9	17.7 ± 0.6	3.5 ± 0.6	19.6 ± 3.1
Insolubles	6.5 ± 4.5	8.4 ± 0.7	4.0 ± 0.7	19.6 ± 1.4

[a] Total uptake: sucrose, 46.6 nmol ^{14}C; 1′-fluorosucrose, 51.3 nmol ^{14}C.
[b] Tissue from the 1 h incubation of corn tissue from Figure 3.
[c] Values are mean recoveries ± SE for three tissue incubations and extractions.

rosucrose. Also, a greater amount of label remained in the insoluble fraction from corn root tissue which had been incubated in [^{14}C]sucrose than from that tissue incubated in [^{14}C](glucosyl)-1′-fluorosucrose, indicating slower metabolism. Soybean leaf discs incorporated little label from either substrate into insoluble material.

DISCUSSION

Although 1′-fluorosucrose is a very poor substrate for invertase, the results of the experiments described above are consistent with its recognition by sucrose carrier proteins. Since either sucrose or 1′-fluorosucrose is capable of competitively inhibiting the uptake of the other into soybean cotyledon protoplasts, both apparently share the same carrier and binding site. The similar pattern of translocation from soybean leaflets also suggests that 1′-fluorosucrose is recognized by sucrose carriers involved in phloem loading in source tissue from these plants.

It has been shown (9) that protoplasts of corn root tissue are not capable of active, carrier-mediated sucrose transport. Labeling patterns observed when asymmetrically labeled sucrose is supplied to corn root segments also suggest that extracellular hydrolysis of sucrose, probably by an invertase, occurs prior to active accumulation of glucose and fructose (6). Sucrose was accumulated by protoplasts, presumably by diffusion, at a rate 3 to 4 times slower than glucose (6). The slow uptake of 1′-fluorosucrose by this tissue is, therefore, consistent with this finding and the inability of extracellular invertase to hydrolyze the sucrose analog to its component hexoses.

The metabolism of 1′-fluorosucrose is slower than that of sucrose in the tissues studied. Although not an invertase substrate, 1′-fluorosucrose is a substrate for sucrose synthetase and may be metabolized to 1′-fluorosucrose and UDP-glucose (data not shown). In the tissues used here, however, a large portion of sucrose metabolism is apparently initiated by an invertase hydrolysis, as evidenced by the low conversion of 1′-fluorosucrose to other metabolites. This selective conversion of the sucrose derivative by two enzymes which occupy parallel pathways for the initiation of sucrose metabolism may be useful to assess the relative contributions of sucrose synthetase and invertase to sucrose metabolism in a variety of tissues.

The kinetics of 1′-fluorosucrose influx by soybean embryo protoplasts also provides some information about the carrier protein interaction with sucrose and the nature of the transport process. Since the carrier protein exhibits a slightly higher affinity for the fluorinated sucrose, the 1′-C and -OH of sucrose must be part of the recognition surface utilized by the protein. The fluorine atom is of very similar size to an OH group and is capable of accepting, but not donating, hydrogen in hydrogen bonds (1). Thus, while H donation by the 1′-OH is apparently required for substrate recognition by invertase, it is not for sucrose synthetase (used in the preparation of 1′-fluorosucrose) or the carrier protein. The slightly enhanced binding (2×) of 1′-fluorosucrose may be due to the 1′-fluorine acting as a more efficient hydrogen bond acceptor in this instance. Alternatively, the tighter binding may result from the more hydrophobic character of fluorine, compared to a hydroxyl group. Recognition and binding of sucrose to taste receptors is largely a hydrophobic interaction (3); and similar binding to the sucrose carrier protein may not be unreasonable.

The membrane transport process can be treated conceptually as being composed of two steps: first, binding of sucrose; and second, its passage through the membrane and release to the cytoplasm. The apparent K_m of the initial rate of ^{14}C-substrate uptake is composed of the following rate constants:

$$S_0 \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C{\cdot}S \xrightarrow{K_3} S_I \quad (1)$$

$$K_m = \frac{K_2 + K_3}{K_1} \quad (2)$$

$$V_{max} = [C]K_3 \quad (3)$$

where K_1 and K_2 are, respectively, the rates of substrate binding and release at the outer membrane surface and K_3 is the rate of substrate release from the carrier protein to the inner surface. Since the apparent V_{max} for uptake is very similar for both sucrose and 1′-fluorosucrose, K_3 is not altered by a substitution which decreased K_2/K_1 by a factor of two. For this carrier, therefore, the rate-limiting steps in the transport process appears to be the movement of sucrose through the membrane-carrier system and its release at the inner plasmalemma surface. If the affinity of sucrose for the carrier binding site is reduced or eliminated by a protein conformational change when the carrier is in the 'release' position, as has been shown for glucose carriers in erythrocytes (2), then the actual transport step may be rate limiting. An altered substrate binding at the internal surface seems a likely characteristic of carrier proteins which are capable of accumulating solutes against a concentration gradient; however, direct testing of binding at the internal surface is not possible using the protoplast system and currently available substrates.

Further single substitutions at hydroxyl groups within the sucrose molecule on both the fructosyl and glucosyl moieties should further define the requirements for substrate binding to sucrose carrier proteins. Properly placed substitutions may also provide other metabolically inert sucroses for use in studying sucrose metabolism and in characterizing the nature of the transport process at the level of carrier proteins.

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