Sugar Uptake by Maize Endosperm Suspension Cultures

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ABSTRACT

Maize (Zea mays L.) endosperm suspension cultures are a useful model system for studying biochemical and physiological events in developing maize endosperm. In this report, sugar uptake by the cultures is characterized. Uptake of 14C-labeled fructose and l-glucose was linear with time, while the rate of uptake of radioactivity from sucrose increased over a 120 min period. Both saturable and linear components of uptake were observed for fructose, glucose, sucrose, 1'-deoxy-1'-fluorosucrose, and maltose. Uptake of mannitol, sorbitol, and l-rhamnose took place at lower rates and was linear with concentration. Rates of incorporation of radioactivity from fructose and glucose exceeded that of sucrose at all concentrations tested. Kinetics of 1'-deoxy-1'-fluorosucrose uptake indicated that 14C from sucrose can be taken up by a saturable carrier of intact sucrose as well as by invertase hydrolysis and subsequent uptake of hexoses. Cell wall invertase was demonstrated histochemically. Further study of fructose uptake at a concentration at which the saturable component predominated revealed sensitivity to metabolic inhibitors, respiratory uncouplers, the nonpermeant sulfhydryl reagent p-chloromercuribenzenesulfonic acid, and nigericin. Uptake was not affected by valinomycin plus K+ and was stimulated by fusicoccin. Fructose and glucose uptake was not pH-sensitive below pH 7.0, whereas uptake of radioactivity from sucrose and 1'-deoxy-1'-fluorosucrose declined as the pH was increased above 5.0. Fructose uptake was not completely inhibited by glucose and vice versa, suggesting the presence of specific carriers. These results indicate that maize endosperm suspension cultures (a) absorb fructose via a typical, energy-requiring, carrier-mediated proton cotransport system; (b) possess saturable carriers for glucose and sucrose; and (c) also absorb sucrose via hexose uptake after sucrose hydrolysis by extracellular invertase.

Photosynthate enters the developing grain of maize (Zea mays L.) via the phloem in the form of sucrose (22). The hydrolysis of sucrose to glucose and fructose, as well as the presence of invertase in the pedicle, has been established (6, 23, 25). The presence of basal endosperm transfer cells suggests that sugars are taken up into the endosperm from the pedicle free space, and microautoradiography revealed symplastic sugar movement through the endosperm (7). Short-term experiments with excised kernels showed passive sugar uptake (10), but neither sustained incorporation of external sugars into starch nor kernel growth has been shown with that experimental system. Although sucrose is hydrolyzed before or during uptake, this hydrolysis does not appear to be essential for sucrose movement into the endosperm (20). Thus, the contribution of invertase to the transport process is uncertain, and the mechanism of sugar transport into intact endosperm and its possible regulation is unclear.

Stable suspension cultures derived from maize endosperm isolated 10 d after pollination have been used as a model system to study endosperm physiology and biochemistry (24). The differentiation of the suspension culture cells into storage cells corresponding to the intact endosperm is evidenced by their ability to synthesize and accumulate starch (2), zein (27), and anthocyanins (19). There are also ultrastructural similarities between the cultures and the intact parent tissue (8). Endosperm suspension cultures generally are supplied with sucrose as the principal carbon source (24), despite the fact that they grow about as well when supplied with glucose or fructose instead of sucrose (28). This study was undertaken to characterize in detail sugar uptake by maize suspension cultures. The lack of susceptibility of the sucrose analog 1'-deoxy-1'-fluorosucrose (FS1) to invertase hydrolysis (12) was exploited to distinguish between uptake of intact sucrose and that of hexoses liberated by cell wall invertase.

MATERIALS AND METHODS

Suspension Culture Establishment and Maintenance. Endosperms were excised from grains of maize (Zea mays L.) inbred A636 harvested 10 d after pollination and cultured on solidified medium containing Murashige and Skoog salts, 30 g/L sucrose, 2 g/L L-asparagine, 0.4 mg/L thiamine HCl, and 6 g/L agar, adjusted to pH 5.6 before autoclaving. After approximately 60 d, the friable callus that had formed was transferred to liquid medium prepared as above, but without agar. Tissue was maintained in 250-mL Erlenmeyer flasks containing 85 mL of medium on a gyrotary shaker at 29°C in the dark and was subcultured every 14 d. Sugar uptake experiments were conducted in a tissue line that had been in culture for about one year. This tissue line was similar to others reported in the literature (2, 27) in that the tissue contained starch and zein (data not shown). Cultures increased from 3 to 16 g fresh weight during the culture period. All uptake experiments were conducted during the mid-log phase of growth (6–8 d after transfer).

Protocol for Uptake Experiments. Tissue was rinsed with three changes of sugarless culture medium (pH after autoclaving was 5.2) and incubated in a fourth change for 6 to 8 h immediately before use. The purpose of this preincubation was to remove all free space sugars and ensure a metabolic demand for extracellular sugars. After the preincubation period, the tissue was passed through an 18-mesh screen to eliminate tissue clumps larger than 1 mm in diameter and collected on a paper towel. Tissue was incubated in 20-mL glass scintillation vials for 60 min (unless otherwise indicated) on a gyrotary shaker at 29°C in the dark. Approximately 0.2 g fresh weight of tissue was placed into each vial, and 1 mL of incubation medium containing 14C-labeled sugar (approximately 1 μCi/mmole) was added. After the incubation period, the medium was removed with a disposable pipette, and the tissue was rinsed three times with 2 mL of

1 Abbreviations: FS, 1'-deoxy-1'-fluorosucrose; CCCP, carbonyl cyanide m-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; PCMBs, p-chloromercuribenzenesulfonic acid.

Received for publication April 29, 1988.
sugarless medium. The tissue was transferred to 50 mL of sugarless medium for 15 min and then collected on preweighed ashless filter paper with a Büchner funnel. Total rinsing time was 30 to 40 min. In preliminary experiments it was determined that slight variations in rinsing times over the range encountered did not affect the amount of radioactivity recovered in the tissue (data not shown). The tissue was air dried overnight, weighed, and oxidized in a Packard\textsuperscript{2} model 306 sample oxidizer. Radioactivity was determined by liquid scintillation spectroscopy.

To determine the rate of loss of radioactivity from the tissue due to leaching or respiration of \(^{14}\)C taken up, tissue was incubated 1 h in 300 mm \([^{3}\text{H}]\)fructose, rinsed according to the standard protocol, and then post-incubated in rinse medium for various times before collecting on filter paper.

In a preliminary experiment, fructose uptake (20 mm) decreased slightly with increased mannitol concentration in the incubation medium (data not shown). Because of the possibility of an osmotic effect on uptake, the total sugar and mannitol concentrations for each treatment of the kinetic experiments was adjusted to 300 mm. For these experiments, tissue was preincubated in sugarless medium containing 100, 200, and 300 mm mannitol for 2 h each in place of the standard preincubation. For determination of the saturation kinetics of mannitol uptake, sorbitol was used as the balancing osmoticum.

For inhibitor experiments, tissue was preincubated for 30 min in sugarless medium containing the inhibitor. Treatment and control media were adjusted to the same ethanol concentration (0.1 to 0.3\%) for those chemicals requiring ethanol as a solubilizing agent. For sugar competition experiments, the total molarity of \(^{14}\)C-labeled sugar, competing sugar, and mannitol was 300 mm for each treatment. To test the possibility that reduction of metabolic demand for sugars was the basis for the noncompetitive inhibition observed, tissue was preincubated in various concentrations of unlabeled glucose for 6 h in place of the preincubation in sugarless medium, then incubated in \([^{14}\text{C}]\)fructose medium as usual. Glucose was not included in the incubation medium.

Invertase Histochemistry. Tissue was rinsed in sugarless medium for 6 h, and then the medium was removed. Tissue was frozen at -70°C, thawed, rinsed with water, and the freeze-thaw-rinse cycle repeated twice. This procedure was necessary to remove all endogenous sugars, which interfere with the staining procedure. The reaction mixture, based on the glucose oxidase reaction, has been described previously (6). Two control treatments were used: sucrose omitted and sucrose replaced with FS, a sucrose analog almost totally resistant to hydrolysis by invertase (12). After incubation for 60 min, tissue was rinsed with water, post-fixed overnight in 1\% (v/v) formalin, and held in 30\% ethanol until photographed.

Chemicals. Murashige and Skoog salts were obtained from Gibco (Grand Island, NY); other reagents except FS were from Sigma. \([^{1}\text{C}]\)Sucrose and \([^{14}\text{C}]\)Fructose were purchased from NEN Research Products (Boston, MA), \([^{1}\text{C}]\)Glucose, UDP-\([^{1}\text{C}]\)Glucose, and \([^{14}\text{C}]\)Maltose were purchased from Amersham (Arlington Heights, IL).

Preparation of FS. FS was prepared by the procedure of Card and Hitz (1). \([^{1}\text{C}]\)Glucosyl]FS was prepared by reaction of UDP-\([^{1}\text{C}]\)Glucose with 1'-deoxy-1'-fluorofructose via sucrose synthase (JC Goodwin, CG Crawford, DC Doehlert, in preparation). The preparation was greater than 98% radiochemically pure as determined by radioscanning TLC using \([^{14}\text{C}]\)glucose and \([^{14}\text{C}]\)sucrose as standards.

\textbf{RESULTS}

The time courses of uptake of radioactivity from fructose, sucrose, and \(\text{L-glucose}\) are shown in Figure 1. With a sugar concentration of 20 mm in the external medium, uptake of fructose and \(\text{L-glucose}\) was linear with time over the experimental periods of 90 and 120 min, respectively. There was no apparent lag phase, and the data could be extrapolated through zero. The rate of \(^{14}\)C incorporation from sucrose increased slightly over the course of 120 min, the data fitting a second order equation \((r = 0.999)\) better than a linear equation \((r = 0.983)\).

Loss of radioactivity from the tissue due to respiration or leaching was estimated by holding tissue in rinse medium for various times after the free space rinse following incubation in 300 mm fructose for 60 min. The loss of \(^{14}\)C was linear over a 3 h period, and the rate of loss was 6% of the total \(^{14}\)C taken up per hour. This slow rate indicated that the tissue was intact and that respiratory loss of \(^{14}\)C over the 30-min rinsing period of the standard protocol was small in relation to the rate of uptake. Of the radioactivity taken up from 300 mm fructose in 60 min, 19\% was removed during the free space rinse, with 81\% remaining in the symplast.

Saturation curves for uptake of \(^{14}\)C from fructose, \(\text{D- and L-glucose, sucrose, FS, maltose, sorbitol, and mannitol are shown}\) in Figure 2. Fructose and \(\text{D-glucose uptake}\) was much higher than uptake of label from the other compounds at all concentrations tested. Fructose and \(\text{D-glucose uptake}\) had both saturable and linear components, the linear component predominating above 150 mm (Fig. 2A). Uptake of \(^{14}\)C from sucrose and FS was also biphasic (Fig. 2B), although the rate of \(^{14}\)C uptake for sucrose exceeded that for FS at all concentrations tested. \(\text{D-glucose uptake}\) had both saturable and linear components, the linear component predominating above 150 mm (Fig. 2A). Uptake of \(^{14}\)C from sucrose and FS was also biphasic (Fig. 2B), although the rate of \(^{14}\)C uptake for sucrose exceeded that for FS. Uptake of \(^{14}\)C from maltose showed a pattern similar to sucrose although the \(V_{\text{max}}\) was much lower (Fig. 2B). \(\text{L-Glucose, mannitol, and sorbitol uptake}\) was linear with concentration over the range 0 to 300 mm (Fig. 2C).

Kinetic constants of the saturable components of glucose and fructose uptake were determined by subtracting the linear component (estimated by \(\text{L-glucose uptake}\)) from the biphasic uptake curves (16) and analyzing the resulting data by iterative curve-fitting using nonlinear regression (9). The saturable component of fructose uptake had a \(K_{m}\) of 60 mm and \(V_{\text{max}}\) of 170 nmol/mg/h, while glucose uptake had a higher \(K_{m}\) (146 mm) and lower \(V_{\text{max}}\) (117 nmol/mg/h). Kinetic constants for sucrose transport

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Time course experiment showing uptake of radioactivity from 20 mm \(^{14}\)C-labeled fructose, sucrose, and \(\text{L-glucose}\) by maize endosperm suspension cultures. Data for fructose and \(\text{L-glucose}\) were analyzed by linear regression; sucrose data were fitted to a second order equation.}
\end{figure}

\textsuperscript{2} Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that might also be suitable.
were not calculated due to the superimposing effects of invertase kinetics. For FS, there was no basis for subtraction of the linear component contribution to total uptake because no nonhydrolyzable, \(^{14}\text{C}\)-labeled disaccharide was tested that exhibited only linear uptake kinetics.

Fructose uptake was not sensitive to changes in pH from pH 5.0 to 7.0, but decreased 35\% from pH 7.0 to 8.0 (Fig. 3). Glucose uptake showed a similar pattern. Uptake of \(^{14}\text{C}\) from sucrose and FS, however, decreased progressively from pH 5.0 to 8.0, with sucrose being more sensitive to pH than FS. The difference in the degree of pH sensitivity between sucrose and FS is consistent with the view that hydrolysis of sucrose by cell wall invertase is involved with sucrose absorption. The pH experiments were the only ones in which a buffer was included in the incubation medium. The pH of the unbuffered incubation medium during the course of a typical fructose uptake experiment (20 mm) remained within the range 5.2 to 5.8 (data not shown).

A histochemical examination of invertase activity resulted in intense staining with sucrose as a substrate, but essentially no staining with FS as a substrate or with no substrate (Fig. 4). Because of the extensive freeze-thaw-rinse pretreatment, any soluble, intracellular invertase would have leached out through the disrupted membranes. Thus, the staining observed was considered to represent cell wall bound invertase.

The effect of inhibitors, uncouplers, ionophores, and fusicoccin on fructose uptake was tested at a fructose concentration of 20 mm, at which level the saturable component comprised most of the uptake (Fig. 2A). Fructose uptake was sensitive to the metabolic inhibitors NaCN and HgCl\(_2\), as well as the metabolic uncouplers CCCP and DNP (Table I). Inhibition of uptake was dependent on the inhibitor concentration. The nonpenetrating, sulfhydryl group modifier PCMBS inhibited fructose uptake up to 98\% at 10 mm. Uptake was not inhibited by the K\(+\)-specific ionophore valinomycin, but was inhibited up to 32\% by the K\(+\)/H\(+\) exchanging ionophore nigericin. Fructose uptake was increased 21\% by fusicoccin, a stimulator of H\(+\) extrusion.

Inhibition of fructose uptake by glucose was noncompetitive (Fig. 5A), as was inhibition of glucose uptake by fructose (Fig. 5B). To test whether the observed noncompetitive inhibition was caused by reduction of metabolic demand for the transported sugar by the inhibiting sugar, tissue was preincubated in unlabelled 100 or 200 mm glucose prior to assaying \(^{14}\text{C}\)fructose uptake. The same noncompetitive inhibition was seen, even though glucose was not included in the \(^{14}\text{C}\)fructose incubation solution (Fig. 5C). Thus, the noncompetitive inhibition observed was probably due to indirect effects, and the lack of competitive inhibition is consistent with the hypothesis that separate carriers exist for fructose and glucose.

**DISCUSSION**

The absence of a lag phase for hexose uptake (Fig. 1) suggests that diffusion into the tissue clumps was not limiting initial
Table 1. Effect of Inhibitors, Ionophores, and Fusicoccin on Uptake of Fructose by Maize Endosperm Suspension Cultures

Tissue was preincubated in medium containing the substance for 30 min before adding [14C]fructose. In experiments with NaN₃, HgCl₂, CCCP, DNP, and PCMBs, 0.05 mM KCl was used in place of sugarless medium.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Percent of Control ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaN₃</td>
<td>0.1 mM</td>
<td>59.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td>34.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>13.8 ± 0.5</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>0.05 mM</td>
<td>40.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>0.1 mM</td>
<td>16.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>CCCP</td>
<td>10 µM</td>
<td>21.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>50 µM</td>
<td>16.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>100 µM</td>
<td>14.9 ± 0.5</td>
</tr>
<tr>
<td>DNP</td>
<td>0.1 mM</td>
<td>32.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>3 mM</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>PCMBs</td>
<td>1 mM</td>
<td>59.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>18.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>10 mM</td>
<td>99.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>50 mM</td>
<td>96.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>100.4 ± 1.5</td>
</tr>
<tr>
<td>Nigericin</td>
<td>0.1 µM</td>
<td>97.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>82.7 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>5 µM</td>
<td>68.4 ± 0.6</td>
</tr>
<tr>
<td>Fusicoccin</td>
<td>1 µM</td>
<td>107.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>5 µM</td>
<td>120.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>10 µM</td>
<td>120.4 ± 1.0</td>
</tr>
</tbody>
</table>

Uptake of sugars by the cells (15). Linearity with time and the extrapolation through zero suggest that carryover of the incubation medium was negligible. The somewhat curvilinear time course of 14C uptake from sucrose (Fig. 1) may have been due to extracellular invertase increasing the external hexose concentration and hence the net sugar uptake rate over time.

The kinetics for uptake of radioactivity from fructose, D- and L-glucose, sucrose, FS, and maltose indicate both saturable and linear components for all of the sugars except L-glucose. The linear components, predominating at external sugar concentrations above 100 mM, are similar to those observed in many other systems and often interpreted as 'diffusion-like' (14, 29). L-Glucose uptake has been used to estimate the diffusion component of sucrose uptake by protoplasts derived from soybean cotyledons, and to distinguish it from another linear component which was nondiffusive (21). The nature of the linear component was not determined in this study; however, it was noted that the slope of the linear component for monosaccharides was considerably higher than that for disaccharides (Fig. 2). Maltose was included in the study for comparison with sucrose uptake. Maize endosperm suspension cultures secrete into the medium an array of vacuolar acid hydrolases, including α-glucosidase, which would cleave maltose to glucose (17). The magnitude of the saturable component of maltose uptake was much lower than the difference between sucrose and FS uptake; thus it is likely that the maltose-degrading enzyme activity is lower than that of invertase. The observation that the slopes of the linear components of the monosaccharides tested are similar to each other and higher than the slopes of the disaccharides as a group suggests that it would be inappropriate to use L-glucose uptake as an estimator of the linear component for disaccharide uptake.

![Figure 5](https://example.com/fig5.png)

**Fig. 5.** Noncompetitive inhibition (A) of fructose uptake by glucose and (B) of glucose uptake by fructose. Panel C shows the effect of preincubating the tissue in various concentrations of nonradioactive fructose for 2 h before incubation in [14C]fructose solutions (glucose was not included in the incubation solution during [14C]fructose uptake).

The results of the present studies are consistent with the previously demonstrated hydrolysis of sucrose in the growth medium of maize endosperm suspension cultures (2). We have shown directly that cell wall invertase occurs in maize endosperm suspension cultures (Fig. 4). Our results indicate that uptake of 14C from sucrose occurs both by uptake of intact sucrose by a plasma membrane carrier and by uptake of hexoses generated by extracellular invertase activity. FS has been shown to be a poor substrate for invertase (hydrolysis at 10 mM was less than 2% of that of sucrose) (12), although it is recognized by sucrose synthase and by a membrane sucrose carrier in soybean cotyledons (12). Therefore, we interpret the FS saturation curve as a better estimate of sucrose uptake kinetics than the sucrose curve, which represents sucrose uptake plus uptake of hexoses produced by invertase hydrolysis. Similarly, the differences in the pH curves between sucrose and FS may represent the contribution of invertase-catalyzed sucrose hydrolysis to sucrose uptake, since maize cell wall invertase has a pH optimum of 5.0 (6). The linear component of FS uptake appeared slightly higher than that for uptake of 14C from sucrose or maltose (Fig. 2A). A possible explanation for this is that the intracellular metabolism of FS via sucrose synthase would maintain a steeper outside/inside con-
The concentration gradient for FS than would exist for sucrose, since pools of sucrose, glucose, and fructose exist in the cell and pools of FS and 1'-fluorofructose do not.

Uptake of the sugar alcohols mannitol and sorbitol was tested because mannitol was used as the osmotic adjusting agent. Lack of evidence for saturable carriers for these compounds is not surprising, because there is little or no mannitol or sorbitol in the culture medium or the endosperm free space. The slightly higher linear rate of sorbitol uptake may be due to metabolism of sorbitol in the cultures. Although not tested with maize endosperm suspension cultures, a ketone reductase activity was reported to be present in maize endosperm which metabolizes sorbitol but not mannitol (5). A lack of mannitol metabolism in the suspension cultures would minimize the effect of the mannitol supplied as osmoticum on the kinetics of sugar uptake. The similarity in slope of the linear uptake of L-glucose with that of the sugar alcohols supports the idea of a diffusion process more than that of nonspecific hexose transport with respect to L-glucose uptake.

The sensitivity of fructose uptake to the general metabolic inhibitors NaN3 and HgCl2, and to the respiratory uncouplers CCCP and DNP, suggests that the saturable component of fructose uptake is energy-requiring. Nigericin is an ionophore which can exchange H+ and K+ across membranes, whereas valinomycin specifically exchanges K+ (18). Therefore, the sensitivity of fructose uptake to nigericin but not valinomycin may indicate a dependence on a pH gradient and not an electrochemical gradient that would be depolarized by the K+ ionophore. This interpretation depends on the assumption that in this short-term experimental system, the ionophores affected primarily events at the plasmalemma and not primarily respiration via effects on mitochondrial membranes. The lack of effect of valinomycin supports that assumption. Fructose uptake was stimulated by fusicoccin, a phytotoxin which stimulates H+ extrusion via a plasmalemma ATPase. This, along with the decreased uptake of fructose at higher pH values (Fig. 2) suggests that a hexose-proton cotransport mechanism exists in the cultures. Another similarity between the observed hexose uptake and carrier-mediated sugar uptake described by others is sensitivity to PCMBs. This suggests that the carrier transporting fructose possesses essential external sulphydryl groups which are modified by this nonpermeant sulphydryl reagent.

The maize pedicle free space sugar concentration has been estimated to be in the range of 400 to 600 mm range (26). It is reasonable to assume that hexoses comprise a major portion of that apoplastic pool in light of the distribution of sugars typically found there (11, 23) and the presence of cell wall invertase (6, 25). Such high sugar levels in the vicinity of the basal endosperm cells would correspond to concentrations well into the linear phase of uptake observed in the cultures. However, the saturable components of sucrose, glucose, and fructose uptake contribute substantially to the rates of uptake at the higher concentrations, and therefore the carrier-mediated transport, although saturated at relatively low concentrations, is not insignificant.

Hydrolysis of sucrose by suspension cultures has been observed in many species including carrots (4, 13, and references therein), but it does not necessarily follow that sucrose hydrolysis is required for uptake by the parent tissue (e.g. intact carrot discs) (3). Examples of both hydrolysis and nonhydrolysis of sucrose during import by various sink tissues abound, and consequently no generalization can be made as to the rationale for hydrolysis in a particular system. It has been suggested recently by Dai et al. (4), who studied sugar uptake by asparagus cells, that carrier-mediated sucrose transport may operate in concert with invertase secretion to regulate sucrose uptake.

The observations of sugar transport by maize endosperm suspension cultures can not necessarily be extrapolated directly to intact endosperm. It should be noted, however, that unlike many undifferentiated suspension cultures derived from other sources, maize endosperm cultures maintain in several respects a differentiated endosperm phenotype (as delineated in the introduction). They may also bear a resemblance in the sugar uptake mechanism. Specifically, if carrier-mediated uptake is shown to occur at the plasmalemma of intact maize endosperm cells, a corresponding carrier in the suspension culture cells may be similar enough to warrant the use of the cultures as a tool for the purification and characterization of the carrier.

Acknowledgments—We thank Deborah L. Black and L. Renee Tucker for their excellent technical assistance, Dr. Douglas C. Doehlert for preparing the maize kernel sucrose synthase used to synthesize FS, and Drs. Cadance Lowell and Charles Boyer for critical reading of the manuscript.

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