In Vitro Sugar Transport in Zea mays L. Kernels

I. CHARACTERISTICS OF SUGAR ABSorption AND METABOLISM BY DEVELOPING MAIZE ENDOSPERM

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STEPHEN M. GRIFFITH, ROBERT J. JONES*, AND MARK L. BRENNER
Department of Agronomy and Plant Genetics (S.M.G., R.J.J.) and Department of Horticultural Science and Landscape Architecture (M.L.B.), University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Short-term transport studies were conducted using excised whole Zea mays kernels incubated in buffered solutions containing radiolabeled sugars. Following incubation, endosperm was removed and rates of net ¹⁴C-sugar uptake were determined. Endogenous sugar gradients of the kernel were estimated by measuring sugar concentrations in cell sap collected from the pedicel and endosperm. A sugar concentration gradient from the pedicel to the endosperm was found. Uptake rates of ¹⁴C-labeled glucose, fructose, and sucrose were linear over the concentration range of 2 to 200 millimolar. At sugar concentrations greater than 50 millimolar, hexose uptake exceeded sucrose uptake. Metabolic inhibitor studies using carbonyl cyanide-m-chlorophenylhydrazone, sodium cyanide, and dinitrophenol and estimates of Q₁₀ suggest that the transport of sugars into the developing maize endosperm is a passive process. Sucrose was hydrolyzed to glucose and fructose during uptake and in the endosperm was either reconverted to sucrose or incorporated into insoluble matter. These data suggest that the conversion of sucrose to glucose and fructose may play a role in sugar absorption by endosperm. Our data do not indicate that sugars are absorbed actively. Sugar uptake by the endosperm may be regulated by the capacity for sugar utilization (i.e. starch synthesis).

The mechanisms of sugar transport through developing maize kernels have not been fully characterized. Most of the current knowledge is based on indirect evidence from young kernels between 20 to 22 DAP⁷ (1, 16, 17, 19). Photoassimilates arriving at the kernel's base presumably move from the phloem throughout the pedicle-parenchyma by symplastic transport (1). Without symplastic continuity between the pedicel and endosperm, sugar (primarily sucrose) must be released into the apoplast of the placento-chalazal region before absorption by the endosperm (1, 8, 13). Porter et al. (13) recently showed that in situ sucrose efflux from the pedicle-parenchyma into an agar matrix was unaffected by treatment with metabolic inhibitors or changes in external pH. This evidence suggests a passive or nonenergy dependent mechanism for sugar release from the pedicel.

The mechanism of sugar absorption by the developing endosperm is unresolved. However, Shannon and co-workers hypothesized that sucrose is cleaved to glucose and fructose by acid invertase in the pedicel and the reducing sugars are preferentially absorbed by the BEC (17, 19). After movement into the endosperm, glucose and fructose are presumably resynthesized into sucrose for temporary storage before conversion into starch (17).

The BEC is morphologically unique compared to the adjacent starch storing parenchyma cells (1, 8). They are often referred to as transfer cells, because of the extensively ingrow cell wall system and abundant mitochondria (1, 8). The BEC may facilitate sugar transport into the endosperm during the linear phase of starch deposition (1), despite the fact that BEC are crushed by the enlarging embryo around 32 to 40 DAP (8). Direct physiological evidence of the involvement of the BEC in sugar transport is lacking. Since processes involved in assimilate transport into the maize endosperm may limit kernel dry matter accumulation (18), understanding the mechanism of sugar absorption by the developing endosperm is important. The objective of this investigation was to characterize the mechanism of in vitro sugar uptake and metabolism by the endosperm in terms of metabolic energy requirements, sugar preference, and patterns of sugar metabolism.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Maize plants (Zea mays L. cv W64A × A619) were grown in the field and greenhouse. Greenhouse plants were grown in 25-L pots at 28°C/25°C, 8/16 h day/night photoperiod, watered daily, and given nutrient solution (Peters 20-20-20) weekly. Daylight was supplemented and extended to 16 h (0500-2100) using high pressure sodium vapor lamps with a quantum flux density of 1200 μE m⁻² s⁻¹. All plants were self-pollinated.

Sugar Transport. The mechanism of sugar transport into the endosperm was characterized using whole excised kernels. Ears were harvested between 20 to 25 DAP and kernels excised from the midcob region. To facilitate apoplastic diffusion of solute to the basal endosperm during in vitro incubation, the lower pedicle containing the vascular region, was trimmed back to the pedicel-parenchyma tissue before the equilibration step. Utilizing this approach lessened tissue damage at the site of sugar transfer and left pedicle invertase, which is thought to be associated with the sugar absorption process, intact. Two kernels per replicate were equilibrated for 30 min in 5 ml of aerated buffered osmotic containing 10 mM MgCl₂ (adjusted to pH 5.5 with NaOH), non-radiolabeled sugar, and a sufficient amount of mannitol to adjust the solution to 200 mOsmol. Following the equilibration step, kernels were incubated for 2 h in a radiolabeled sugar (0.55 μCi ml⁻¹) solution of similar composition to the equilibration buffer. The metabolic inhibitors CCCP, DNP, and NaCN were used to

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²Abbreviations: DAP, days after pollination; BEC, basal endosperm cells; CCCP, carbonyl cyanide-m-chlorophenylhydrazone; DNP, 2,4-dinitrophenol.
determine the role of metabolic energy on sugar transport. These inhibitors were introduced at the beginning of the equilibration period and their presence continued throughout incubation in 

\[ ^4C \text{-labeled sugar} \]. Uptake of radiolabeled sugar was terminated by three washes with solution (similar in composition to the equilibration buffer) of 2, 3, and 5 min duration, respectively, to remove free space radiolabeled sugars. The wash intervals were predetermined by efflux analysis adapted from Gifford and Thorne (3) using \([1,2-^3H] \text{PEG} (4000) \) and \([^{14}C]\)glucose. Following the final wash, endosperms were removed from kernels (about 250 mg fresh weight each). Two endosperms per replicate were digested in 1 ml of HClO\(_2\), H\(_2\)O (3:2, v/v) at 50°C until the solution was devoid of color (about 3 h). The endosperm samples were cooled and H\(_2\)O:Aquasol-2 (3:8, v/v) (New England Nuclear) was added. Radioactivity was determined by liquid scintillation spectrometry.

**Pathway of in Vitro Sugar Movement.** To verify that \([^{14}C]\)-sugar uptake into the endosperm was via the pedicle, three methods of incubation were compared: (a) whole kernel immersion; (b) pedicle immersion only with kernel upright; and (c) inverted distal-half of the kernel immersed. Following incubation with 10 mM \([^{14}C]\)glucose for 2 h, kernels were separated into various subcomponents, and the total \([^{14}C]\)accumulated in each component was determined.

**Sugar Analysis.** Endogenous sugar concentrations and osmolality were determined from cell sap obtained from freeze-thawed kernel components (i.e. pedicle, endosperm, and embryo). Kernel components were placed in liquid N\(_2\), removed and thawed, then centrifuged at 3,000g for 15 min at 4°C. Aliquots of sap were then assayed for: (a) sugars, using a Waters HPLC system (Waters Associates) fitted with a carbohydrate analysis column and an Erma 7510-R1 detector (Erma Optical Works Ltd); and (b) total solute osmolality, measured with a vapor pressure osmometer (Campbell Instr. model No. 5500).

**Enzyme Assay.** Soluble and insoluble acid invertase (EC 3.2.1.26) activities were determined as described by Giaquinta et al. (2) with slight modification. Ground, freeze-dried samples were added to 1 ml of 85 mM Hepes (pH 8.0), 15 mM DTT, and 15 mM EDTA. Samples were then centrifuged at 20,000g for 20 min at 4°C. The supernatant was passed through a Pharmacia Sephadex G-25 M column equilibrated with 5 mM Hepes (pH 7.0). Aliquots of the eluted crude-enzyme extract or the washed (three times with extraction buffer) insoluble fraction were assayed for invertase at 30°C for 30 min in 50 mM sucrose. The reaction solution was buffered to pH 4.7 with 100 mM sodium acetate. Reducing sugars were quantified using a procedure of Nelson (10).

**Metabolism of Exogenously Supplied Sugar.** Metabolism of \([^{14}C]\)-sugar by endosperms was determined following whole kernel incubation with \([^{14}C]\)-sugar as described above. Kernels were sampled at 2.5 and 50.0 h and the endosperms were isolated for the determination of net incorporation of \([^{14}C]\)-sugar into soluble and insoluble fractions. Radiolabeled endosperm tissue was extracted with 5 ml of 80% (v/v) ethanol heated to 70°C for 2 h, and allowed to stand for 8 h at 4°C. The samples were then ground using a glass rod and each washed with 2 ml four rinses of 80% (v/v) ethanol. Samples were centrifuged at 3,000g for 5 min after each rinse and the ethanol-soluble and insoluble fractions were saved. Labeled \([^{14}C]\)-sugars were separated by HPLC, sugar peaks collected, and radioactivity of each peak quantified by liquid scintillation spectrometry as described previously (5). The incorporation of radiolabel into insoluble matter was determined after tissue digestion as described above.

**RESULTS**

**Kernel Component Sugar Concentration and Distribution.** Endogenous sugar concentrations were measured to determine the sugar gradients between the pedicel and endosperm and embryo (Fig. 1). Fructose, glucose, and sucrose were the major sugars extracted from the maize kernel pedicel, endosperm, and embryo at 25 DAP. In the zone of endosperm sugar absorption (i.e. pedicel-parenchyma/placenta-chalazal/basal endosperm), the pedicel-parenchyma contained the highest total sugar concentration (i.e. sum of fructose, glucose, and sucrose), 415 mM, while the lower endosperm zone had only 238 mM. In addition, the pedicel-parenchyma, a tissue high in soluble and insoluble acid invertase activity (Table I), contained elevated concentrations of reducing sugar, 259 mM, compared to each of the lower, middle, or upper endosperm zones of 100, 30, and 20 mM, respectively (Fig. 1).

The sucrose concentration declined gradually from the pedicel vascular zone, continuing distally through the kernel to the middle endosperm region. In some samples the sucrose concentration declined without a dominant peak in concentration in the middle zone.

Sucrose (205 mM) comprised 98% of the total embryo sugar (Fig. 1). Hexoses did not accumulate in the embryo. Detailed characteristics of sugar influx and metabolism by developing embryos are described in an accompanying paper (4).

**Kernel Component Osmolarity.** The osmolarity of cell sap collected from the pedicel, endosperm, and embryo revealed the existing solute (organic and inorganic) concentrations throughout kernel development. A concentration gradient from the pedicel to the endosperm and embryo was apparent throughout kernel ontogeny (Fig. 2). The greatest difference in solute concentration existed between the pedicel and endosperm and was largest during linear dry weight accumulation (about 20–40 DAP). At 25 DAP sugars comprised a large proportion of the

![Fig. 1. Distribution of endogenous sucrose (■), glucose (●), and fructose (□) concentrations within the lower pedicle vascular region (P Vs), pedicel-parenchyma (PP), lower endosperm (L En), middle endosperm (M En), upper endosperm (U En), and embryo (Emb) at 25 DAP. Sugars were assayed from cell sap using HPLC analysis (see "Materials and Methods"). Values represent means of duplicate samples; vertical bars represent 1 SE.](image)

**Table 1. Soluble and Insoluble Invertase Activity of Various Maize Cob and Kernel Components at 20 DAP**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Acid Invertase Activity</th>
<th>Soluble</th>
<th>Insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cob</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pith</td>
<td>20 ± 1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>22 ± 2</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Pedicel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular-pedicel</td>
<td>7 ± 1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Spongy-parenchyma</td>
<td>31 ± 3</td>
<td>52 ± 6</td>
<td></td>
</tr>
<tr>
<td>Pedicel-parenchyma</td>
<td>79 ± 9</td>
<td>134 ± 12</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Values of osmolarity measured from cell sap extracted from the pedicel, endosperm, and embryo maize kernel components. Kernels were harvested from field grown ears of maize at various days after pollination. Values represent the means ± 1 se of three replicates.

Table II. Accumulation of [14C]Glucose into Specific Kernel Subcomponents Resulting from Two Different in Vitro Kernel Incubation Methods: Whole Kernel Immersion (Whole) and Pedicel Immersion Only with Kernel Upright (Basal)

Each value is the mean ± 1 se of two replicates.

<table>
<thead>
<tr>
<th>Kernel Component</th>
<th>Incubation Method</th>
<th>Whole</th>
<th>Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedicel</td>
<td></td>
<td>75 ± 15</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>Endosperm</td>
<td></td>
<td>38 ± 8</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Lower-third</td>
<td></td>
<td>10 ± 3</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Middle-third</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Upper-third</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryo</td>
<td>Lower-half</td>
<td>22 ± 4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td></td>
<td>Upper-half</td>
<td>5 ± 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>150 ± 25</td>
<td>149 ± 21</td>
</tr>
</tbody>
</table>

solute potential in each component (Fig. 1).

Pathway of in Vitro Sugar Movement into the Excised Kernel. Sugar uptake, using immersion of whole kernel or pedicel only, revealed sugar flux from the pedicel into the endosperm and embryo (Table II). The distal immersion treatment showed no indication of [14C]glucose entering the endosperm or embryo through the pericarp.

Transport Kinetics. The uptake of radiolabeled sucrose, glucose, and fructose by the endosperm was relatively linear over the concentration range of 2 to 200 mM (Fig. 3). Rates of sucrose uptake did not differ significantly from glucose and fructose uptake until exogenous sucrose concentrations exceeded 50 mM. Above 50 mM fructose and glucose uptake was approximately twice that of sucrose uptake, based on equal molar amounts of each sugar, but did not differ on a hexose equivalent basis.

Role of Metabolic Energy. Treatment with CCCP at 5 μM and DNP and NaCN at 1 mM had no effect on 5 mM sucrose, glucose, and 3-O-methyl glucose (3-O-MG) uptake into the endosperm during a 2-h incubation period (Table III).

Short-term effects of temperature on sugar uptake were examined to further evaluate the role of cellular metabolism in sugar transport. One mM glucose and 3-O-MG uptake at 20 and 30°C gave calculated Q10 values of 1.1 and 1.2, respectively. The optimum temperature for glucose uptake was 35°C. At 4°C, glucose uptake, from a solution containing 1 mM glucose, was reduced 58% (0.26 ± 0.01 nmol h⁻¹-endosperm) over the 25°C control (0.62 ± 0.02 nmol h⁻¹-endosperm).

Endosperm Metabolism of Accumulated Radiolabeled Sugars. The interconversion of radiolabeled sugars by the endosperm...
the $^{14}$C originally labeled in the glucosyl moiety of asymmetrically labeled sucrose was recovered in the glucose and fructose fraction. As time progressed more of this label appeared in the sucrose pool, similar to that observed for the labeled hexose treatments. Experiments utilizing asymmetrically labeled sucrose (glucosyl moiety labeled only) served to test the extent of free space sucrose hydrolysis. Free space hydrolysis of asymmetrically labeled sucrose would cause cytoplasmically synthesized sucrose to become randomly labeled from isomerase action upon intracellular hexose. Results showed that the $^{14}$C fructose/glucose ratio of endogenously labeled sucrose, following uptake of $[^{14}C]$ (glucosyl)sucrose (2 h), was 0.81.

Although hexoses appear to be taken up at higher rates than sucrose in maize endosperm (Fig. 3), this may not necessarily result in greater rates of hexose incorporation into starch. To test this we pulse-chased whole excised kernels with $^{14}$C-sugar in vitro for up to 5 h and then measured the incorporation of $^{14}$C into the insoluble fraction. After 2.5 h (1.5-h chase), 0.66, 0.62, and 0.63 nmol equivalents of glucose were incorporated per endosperm into the insoluble fraction from the supplied sucrose, glucose, and fructose, respectively (Table V). After 5 h, there was a trend for more glucose equivalents to be incorporated into insoluble matter from glucose than from fructose or sucrose. None of the values reported in Table V are significantly different from one another.

### DISCUSSION

The in vitro excised whole kernel system enabled us to investigate both sugar uptake and its subsequent metabolism by the endosperm. Utilizing this approach lessened the chance of tissue damage at the site of sugar transfer and left enzymes (i.e. cell wall invertase) which may be involved in the transport process (17) undisturbed.

Maize kernel sugar distributions and concentrations (Fig. 1) were consistent with those previously reported by Shannon (17). The high concentration of total sugar found in the pedicel region, as compared to the adjacent endosperm, indicated that sugar absorption by the endosperm may be slower than the rate of accumulation in the pedicel (17), and thus potentially could be limiting kernel growth (18). Although the BEC's invaginated cell walls may provide increased membrane surface for sugar absorption, sugar uptake rates may still limit endosperm starch synthesis.

Sugar uptake into the maize endosperm was relatively linearly related to exogenous sugar concentration (Fig. 3). A similar linear relationship in other plant systems has been suggested to represent a passive, noncarrier mediated uptake process (9, 12, 15). The sugar concentration gradient from the pedicel-parenchyma, especially for glucose and fructose (Fig. 1), would support a diffusion mechanism. Evidence of a carrier-mediated sugar transport mechanism, indicated by saturation kinetics, was not found (Fig. 3) using our in vitro excised whole kernel system. It must be noted though, that precise interruption of kinetic data derived from using plant material composed of multiple tissue types may be complicated and cannot be extrapolated extensively without further experimentation.

Metabolic inhibitor and temperature studies also suggest that sugar transport into the endosperm involves a passive process. Metabolic inhibitors CCCP, DNP, and NaCN inhibit the saturable component of secondary active sugar transport in plant systems but not the diffusion-like, nonsaturable component (14, 15). Our data support the work of Shannon and Liu (20) on maize endosperm slices in which they showed that although starch synthesis was inhibited, sugar transport was not greatly affected. Thus, at physiological sugar concentrations in the pedicel (Fig. 1), sugar uptake into the endosperm may not involve metabolic energy closely coupled to sugar transport. If the BEC facilitate sugar absorption from the apoplast they may provide additional membrane surface area for sugar absorption during early stages of endosperm development. At later stages the BEC become crushed by the enlarging endosperm and embryo (32-40 DAP) (8) and may no longer function.

Our sugar metabolism studies support Shannon and Dougherty's (19) data, suggesting that sucrose is extracellularly hydrolyzed and intracellularly reconverted into sucrose or starch. It is not known for certain whether sucrose was extracellularly hydrolyzed prior to uptake but the following evidence tends to support free space hydrolysis of sucrose: (a) short-term experiments using asymmetrically labeled sucrose showed significant cleavage of sucrose and subsequent isomerization of its hexose moieties; (b) the pedicel contains a high endogenous hexose/sucrose ratio, while the inverse was observed in the endosperm; and (c) no uptake specificity for fructose, glucose, or sucrose, on an hexose basis. In addition, we found a trend for greater amounts of radioiodabeled glucose to be incorporated into insoluble matter (Table V) than from labeled fructose or sucrose, suggesting a more direct pathway through glucose. It has been demonstrated in wheat (6, 14) that although hexose sugar is absorbed by the developing endosperm faster than sucrose, its subsequent incorporation into starch was significantly reduced compared to sucrose. This does not appear to be true in maize endosperm.

We suggest that hexose uptake may be regulated by metabolic processes within the endosperm. Endosperm intracellular sugar utilization and compartmentation may keep endogenous sugar pools at a steady state with the apparent free space of the placental-chalazal region. Continual maintenance of a hexose concentration gradient from the pedicel to the endosperm may arise by sucrose hydrolysis in the pedicel region and resynthesis to sucrose, starch, or other products in the endosperm. Enzymes involved in sucrose synthesis (i.e. sucrose synthase) may play a role in sucrose resynthesis in maize endosperm. It has been shown that sucrose synthase activity remains high during high rates of starch synthesis (11). If sugar conversion to starch or other products in the endosperm is the major regulating factor driving sugar absorption, then additional research is needed to understand these processes and their regulation. There is some indication that the endosperm’s potential for sugar utilization during linear fill is established early during the endosperm cell division phase (7). These questions are currently being addressed in our laboratory.

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