Sugar Content and Activity of Sucrose Metabolism Enzymes in Milled Rice Grain

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ABSTRACT

Most rice (Oryza sativa L.) cultivars grown in the United States were selected for endosperm starch properties and not soluble sugar content. The minor pool of soluble sugar may affect the qualities of rice as a food. Some cultivar variation in soluble sugar content was detected in milled grain, essentially the starchy endosperm, of long grain varieties. Milled grain of cultivars Lemont and Texmati had a soluble sugar content of 0.21 and 0.35% (w/w), respectively, on a fresh weight basis. The dorsal portion of the milled grain contained the greatest amount of soluble sugar, approximately tenfold the amount found in the central core of the grain. Extracts of the milled grain contained sucrose-phosphate synthase (EC 2.4.1.14) and sucrose synthase (EC 2.4.1.13) activities, which were separated by anion exchange chromatography. The presence of sucrose-phosphate synthase in the rice endosperm suggested a mechanism for sucrose accumulation which might be involved in carbon partitioning during grain development.

Mature storage tissues such as cereal endosperm consist primarily of starch and a minor pool of soluble sugar (9, 14, 16). Consequently, the major fate of translocated photosynthate entering the developing endosperm is to be metabolized by invertase or sucrose synthase into precursors for starch biosynthesis. The endosperm sucrose pool represents an alternative storage form for incoming photosynthate. It is unclear whether endosperm sucrose accumulation depends on transport of unmetabolized sucrose into the endosperm cell, or whether resynthesis of sucrose occurs from a pool of hexoses and nucleotide sugars common to the starch biosynthesis pathway (7).

Resynthesis of sucrose might be catalyzed by sucrose synthase, or by a combination of sucrose-phosphate synthase and sucrose phosphatase activities (7). The presence of sucrose-phosphate synthase in many storage organs (4, 6, 10, 12, 16) suggests that some sucrose may be resynthesized using this nonreversible pathway.

Endosperm mutations are known which increase soluble sugar content of cereal grain (13). The increase in sugar in these mutants usually results from a blockage in starch biosynthesis, and consequently, grain weight is reduced. The general observation that crop productivity is not limited by photosynthesis (5), suggests that sucrose content might be increased in cereal endosperm without reducing starch synthesis and grain fill.

Most long grain, or indica type, rice grown in the United States were selected for high amylose starch in order to have flaky texture after cooking. Although rice sweetness may be important in the flavor and color reactions that take place during cooking or processing, little has been done to identify factors which influence sugar content during grain fill. Soluble sugar content of some commercial rices was analyzed here to determine variation in this property. In addition, column chromatography was used to demonstrate that rice endosperm contains both sucrose synthase and sucrose-phosphate synthase. The presence of sucrose-phosphate synthase in the rice endosperm suggests the hypothesis that some sucrose accumulation may be due to resynthesis through this nonreversible pathway.

MATERIALS AND METHODS

Rice Grain

Milled rice grain (Oryza sativa L.) of cultivars Lemont, Rexmont, and Starbonnet were obtained from the Texas Rice Research Foundation at Beaumont, TX. Milled rice grain of cultivar Texmati was obtained from Farms of Texas Co. at Alvin, TX. The milled grain was ground into a flour using a Udy Cyclone mill and stored at −20°C until use.

Samples of rough rice grown in Beaumont, TX, were de-hulled, and the whole caryopsis used for microscopy.

Sugar Content of Milled Grain

Samples (100 mg) of milled grain flour were extracted with 2.5 mL of 70% (v/v) ethanol as described previously (16). Triplicate aliquots of the extracts were analyzed for sucrose using a resorcinol reagent (8, 16), and for soluble reducing sugar using the neurocuprine technique relative to standards of glucose (2).

Milled grain of cultivar Starbonnet was sliced using a scalpel. The top portion and bottom portion averaged 31 and 37% (w/w), respectively, of the total milled grain weight. The middle portion of the grain was sliced into dorsal, lateral, ventral, and core sections. Portions of the milled grain were ground into flour using a mortar and pestle. Flour samples (25 to 35 mg) were extracted twice at 80°C for 10 min with a total volume of 1.2 mL of 70% (v/v) ethanol. Sucrose and soluble reducing sugar content were measured as indicated above.

Microscopy

The rice caryopsis was embedded in low viscosity plastic. Cross-sections of the middle portion of the caryopsis were stained with I₂KI and photographed using a Reichert photomicroscope.
described previously (8, 14). The precipitated extract was resuspended in 2 to 4 mL of 20 mM Hepes-KOH (pH 7.5) and clarified by centrifugation at 36,000 g for 1 h. The concentrated extract was desalted by Sephadex G-25 chromatography and passed through a 0.45 micron filter. This desalted, concentrated extract was used for enzyme assays and for anion exchange chromatography. Aliquots (1–4.5 mL) were loaded onto an anion exchange (Pharmacia Mono Q HR 5/5) column using a 1 mL injection loop and a Perkin-Elmer Series 400 liquid chromatography pump. The flow rate was 0.81 mL/min and 1 min fractions were collected. The elution medium was 20 mM Hepes-KOH (pH 7.5), 10% (v/v) glycerol, and 5 mM dithiothreitol, with a 0.1 to 0.4 M NaCl gradient followed by a step gradient up to 1 M NaCl. In some experiments the elution medium was the same as described previously (15). The shape of the salt gradient was measured using a Radiometer conductivity meter.

Assays for Enzyme Activity

Sucrose-phosphate synthase (UDP-D-glucose: D-fructose 6-phosphatase 2-α-D-glucosyltransferase, EC 2.4.1.14) and sucrose synthase (UDP-D-glucose: D-fructose 2-α-D-glucosyltransferase, EC 2.4.1.13) activities were assayed as described previously (8, 16). The sucrose-phosphate synthase assay medium contained 35 μL of extract, 80 mM Hepes-KOH (pH 7.5), 14 mM MgCl₂, 10 mM fructose 6-phosphate, 1 mM dithiothreitol, and 10 mM UDP-glucose in a 0.1 M volume. The sucrose synthase assay medium was identical except that 80 mM

<p>| Table I. Sucrose-Phosphate Synthase and Sucrose Synthase Activities in Extracts From Milled, Long Grain Rices |</p>
<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Harvest Year</th>
<th>n</th>
<th>Sucrose-phosphate synthase</th>
<th>n</th>
<th>Sucrose synthase</th>
</tr>
</thead>
<tbody>
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<td>Lemont</td>
<td>1985</td>
<td>5</td>
<td>4.7 ± 3.6</td>
<td>3</td>
<td>49.1 ± 6.3</td>
</tr>
<tr>
<td>Lemont</td>
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<td>5.5 ± 3.7</td>
<td>7</td>
<td>92.1 ± 39.8</td>
</tr>
<tr>
<td>Rexmont</td>
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<td>6.8 ± 2.7</td>
<td>1</td>
<td>58.1</td>
</tr>
<tr>
<td>Texmati</td>
<td>1985</td>
<td>2</td>
<td>1.5 ± 0.6</td>
<td>1</td>
<td>55.7</td>
</tr>
</tbody>
</table>
was (SPS) and desalted, concentrated to fructose
replaced Bicine-KOH (pH 8.0) was the buffer and 10 mM fructose
replaced fructose-6-phosphate. Assays were run for 0.5 h at
25 to 30°C. Enzyme activity in column fractions was deter-
mined as nmol sucrose-phosphate or sucrose per 0.5 h per 35
µL of partially-purified extract. The specific activity of the
desalted, concentrated crude extract was calculated on a per
minute basis using determinations based on 0.5 h incubations.
Protein contents of crude extracts and column fractions were
determined with a dye-binding technique (1), using standards
of bovine serum albumin.
Fructose-6-phosphate phosphatase activity was measured
using the sucrose-phosphate synthase assay medium minus
UDP-glucose. Incubations were for 0.5 h. The amount of
phosphate released was measured using 2.5 mL of a phosphate
reagent (18). Time zero phosphate content was determined
by adding the phosphate reagent prior to the grain extract.

Statistical Analysis

Data on sugar content were the result of independent
extractions of grain from the same harvest year, grown in the
same environment. Variance of the mean was calculated as
a standard deviation and statistical differences were analyzed
using the SAS program on a personal computer. The Dun-
can’s Multiple Range Test was used to determine statistical
difference at the 95% level of confidence.

RESULTS

Soluble Sugar Content of Milled Long Grain Rice

Milled rice grain from long grain cultivars had low soluble
sugar content (Fig. 1). The sum of sucrose plus soluble reduc-
ing sugar on a flour fresh weight basis was 0.21% (w/w) for
cultivar Lemont and 0.35% (w/w) for Texmati. The sucrose
values for Texmati rice were high relative to some of the other
long grain varieties grown in the United States, represented
here by Lemont and Rexmont (Fig. 1). The Lemont caryopsis
had the highest sucrose content of 18 long grain rices grown
in Texas in 1984 (16). Texmati was not analyzed then.
The milled grain analyzed here appeared to be starchy
endosperm without significant amounts of the outer grain
tissues. The high sugar content in the outer caryopsis (14, 16)
requires that comparisons of sugar content between rice cul-
tivars be made with grain milled to the same degree.
The sucrose values reported above probably include a small
amount of raffinose. The reagent for sucrose determinations
reacts equally well with raffinose. The ethanol extracts used
for the determinations in Figure 1 were analyzed by liquid
chromatography. The raffinose contribution to the total su-
crose plus raffinose pool was 8, 8, and 15%, respectively for
Lemont, Rexmont, and Texmati (G Cohen, personal
communication).
Soluble sugar was not uniformly distributed throughout
the milled grain (Fig. 2). The dorsal portion of the milled grain,
which would be adjacent to the vascular tissues of the cary-
opsis, had 10 times the amount of sugar found in the center core
of the endosperm. Morphologically, the outer layer of starchy
endosperm cells (subaleurone layer) contained appreciable
cyttoplasmic space as well as amyloplasts, whereas cells in the
center of the starchy endosperm were filled with starch gran-
ules (Fig. 3).

Sucrose-phosphate Synthase and Sucrose Synthase
Activities in Milled Rice Grain

Extracts of milled rice had both sucrose-phosphate synthase
and sucrose synthase activity (Table 1). Sucrose-phosphate
synthase activity in extracts of milled grain was approximately
one-tenth of the activity found in caryopsis extracts (16). The
specific activity of the sucrose synthase activity (Table 1) was
comparable to that found in extracts of the whole caryopsis
(16).
In two experiments, fructose 6-phosphate phosphatase ac-
tivity averaged 29 nmol phosphate (min)−1 (mg protein)−1,
which would produce 0.3 mM fructose at the end of a 30 min
incubation. No allowance was made for any contribution
sucrose synthase made to the measurement of sucrose-phos-
phate synthase activity.
Sucrose-phosphate synthase activity in the milled grain
extract was not due to the presence of scutellar tissues adhering
to the milled grain. In two experiments, the embryo end of
milled Lemont grain was removed prior to making flour from
the grain. The average specific activity of sucrose-phosphate
synthase in these two experiments was 9 nmol (min)−1 (mg
protein)−1, which compares to the value obtained with extracts
from normal milled grain (Table 1).
Sucrose-phosphate synthase was separated from sucrose
synthase by anion exchange chromatography (Fig. 4). Con-
sequently, at least part of the sucrose-phosphate synthase
activity detected in the crude extracts was due to the enzyme,
and not just an artifact based on an interaction between
sucrose synthase and other enzymes in the extract.
Only a portion of the sucrose-phosphate synthase activity
measured in the crude extract was recovered after chromatog-
raphy. In the experiment shown in Figure 4, 25% of the sucrose-phosphate synthase activity loaded on the column was recovered in the indicated peak. Sucrose-phosphate synthase activity was recovered at the indicated elution position in six out of nine experiments with cultivar Lemont, and in the one experiment done with cultivar Rexmont. In three of the experiments with Lemont, none of the sucrose-phosphate synthase activity loaded on the column was recovered after anion exchange chromatography. Sucrose synthase activity was more stable, and the peak indicated in Figure 4 accounted for greater than 80% of the activity loaded on the column for three experiments.

DISCUSSION

The long grain rices grown in the United States generally have a low sugar content (16). Japonica rice mutants are known where endosperm soluble sugar content can reach 6% (w/w), but the grain weight is reduced (13). The 0.4% (w/w) soluble sugar content reported here for Texmati milled rice is low, but does suggest that varietal differences exist in this trait when rice grain with normal starch composition are analyzed.

Rice endosperm can accumulate soluble sugar late in grain fill after starch biosynthesis has decreased (14). The dorsal vascular bundle in the pericarp remains functional late in development (9), and consequently provides photosynthetic endosperm metabolism.

The gradients of soluble sugar in the rice starchy endosperm (Fig. 2) show that sugar has been effectively converted to starch in the central core. It seems likely that sucrose synthase participates in this process (7). The outer starchy endosperm of rice, especially the dorsal region, contains more soluble sugar. Sucrose, glucose, and fructose are the major rice sugars (14, 16), and could accumulate in either the apoplastic outside the cells, or in the cytoplasm. Although amyloplasts occupy a large volume in the starchy endosperm, they have only limited permeability to hexoses and sucrose (3). Furthermore, both sucrose-phosphate synthase and sucrose synthase are cytoplasmic enzymes in castor bean endosperm (11). Consequently, sucrose resynthesis by either enzyme would have to compete for precursors common to starch biosynthesis. The combination of sucrose-phosphate synthase and sucrose-phosphate activities might provide a means of redirecting some carbon flow toward an alternative storage compound in the nonplastid part of the cell.

Sucrose-phosphate synthase activity of milled rice was separated from sucrose synthase by anion-exchange chromatography (Fig. 4). This result supports the hypothesis (6) that both sucrose-phosphate synthase and sucrose synthase function in cereal endosperm metabolism. Increases in sucrose-phosphate synthase activity during development of beetroot (4) or muskmelon (10), or after cold stress in potato tuber (17), were associated with increased sucrose accumulation. Therefore, the amount of sucrose-phosphate synthase activity might regulate sucrose accumulation in storage tissues.

The results here show that endosperm sucrose content can be increased in normal long grain rice through cultivar selection. The question remains whether sucrose-phosphate synthase and sucrose resynthesis are important factors in this character. Sucrose-phosphate synthase activity was uniformly low in extracts of milled rice; but the labile nature of this protein (7) would probably necessitate cultivar comparisons on freshly-harvested endosperms.

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LITERATURE CITED