Phytochemistry, Vol. 22, No. 11, pp. 2513–2515, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

STARCH AND WATER-SOLUBLE POLYSACCHARIDES FROM *SUGARY* ENDOSPERM OF SORGHUM*

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(*Received* 11 *February* 1983)

Key Word Index—*Sorghum bicolor*; Gramineae; sorghum; α-glucan; phytoglycogen; starch.

Abstract—The starch and water-soluble polysaccharides from *sugary* (*su*) endosperm of sorghum were isolated and characterized. Starch granule structure and composition were similar to *sugary* maize starch. The water-soluble polysaccharide was compared to rabbit liver glycogen and *su* maize phytoglycogen. Based on iodine spectra, conversion to maltose by β-amylase, average chain length, size distribution of chains after isoamylase debranching and resistance to pullulanase debranching, *su* sorghum water-soluble polysaccharide was concluded to be phytoglycogen. Particulate and soluble fractions of *su* sorghum phytoglycogen were prepared by centrifugation at 14 000 *g*. These fractions were similar to fractions of *su* maize phytoglycogen prepared by the same method. These results support the hypothesis that *su* maize and *su* sorghum are homologous mutants.

INTRODUCTION

The water-soluble polysaccharide phytoglycogen was reported to be a major component of the endosperm of sweet corn (homozygous for the *sugary*, *su*, gene) in 1939 [1]. Later studies demonstrated the similarities of glycogens from other species and maize phytoglycogen [2–5]. Recent reports have shown that phytoglycogen is present in particulate fractions as well as soluble fractions [6, 7]. Phytoglycogen is an unusual polysaccharide in higher plants and has been observed only in the Mullerian bodies of *Cecropia peltata* and in *sugary* maize [8]. Karper and Quimby [9] reported a gene in sorghum which they termed *sugary* (*su*). Because endosperms of sorghum homozygous for the *su* gene were higher in sucrose [9] and contained significant levels of water-soluble polysaccharide [10], Karper and Quimby [9] suggested that the *su* gene of sorghum was homologous to the *su* gene of maize.

A large number of examples of homologous mutations in starch biosynthesis have been reported. The most common mutants are *waxy* types (the starches contain no amylose but 100% amylopectin) and high amylose starch types (see review [11]). The conclusion that the *su* mutation of sorghum is homologous to the *su* mutation of maize would be strengthened by a more complete characterization of the water-soluble polysaccharide to ascertain whether indeed it is phytoglycogen. In this report, we demonstrate that the water-soluble polysaccharide from *su* endosperms of sorghum is phytoglycogen and is similar in structure to *su* maize phytoglycogen.

RESULTS AND DISCUSSION

The phenotypes of the seeds of four accessions of sorghum reported to be *sugary* were found to be quite variable. The phenotype of seeds of IS4668 was glassy, small seeded as previously described for *su* sorghum [9]. The other three accessions appeared to be heterogeneous mixtures of small glassy seeds and larger full seeds. Extracts from seeds of all four accessions contained significant levels of water-soluble polysaccharides (Table 1). However, seeds of IS4668 contained 6–13 times more water-soluble polysaccharide than the other three accessions. Since these accessions were open-pollinated, it is not possible to exclude cross-pollinations with non-sugary lines. Therefore, although the structural features of the water-soluble polysaccharides from all four accessions were similar, only the properties of the α-glucans from IS4668 will be reported in detail. The starch granules from *su* sorghum were a mixture, including very small, compound granules, small simple granules and large spherical or polygonal granules (not shown). Similar ranges of types have been reported for starch granules isolated from *su* maize endosperm [12]. Fractionation of dispersed starch on agarose columns resulted in two peaks (Fig. 1). The early eluting peak (column fractions 10–20) was amylopectin, while the later eluting peak was amylose. The proportion of amylose was higher than the 25–30% amylose in non-mutant starches. The proportion of amylose was higher in starch from IS4668 seeds than

Table 1. Per cent composition of the endosperm carbohydrates of seeds of four sorghum accessions carrying the *sugary* gene

Accession	Starch	Water-soluble polysaccharide	Total
IS4526	37	1.0	38
IS4577	48	2.3	50
IS4668	22	13.2	35
IS5029	52	1.7	54

*Contribution No. 20, Department of Horticulture, The Pennsylvania State University. Authorized for publication No. 6593 in the journal series of the Pennsylvania Agricultural Experiment Station.

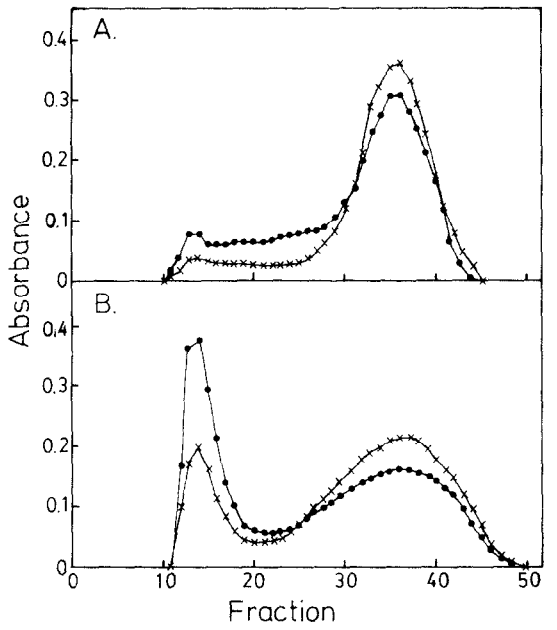

Fig. 1. Sepharose-CL-2B gel filtration of starches isolated from *sugary* sorghum endosperm. A, IS4668; B, IS4577. Fractions were assayed with iodine; A_{540} (●), A_{660} (×).

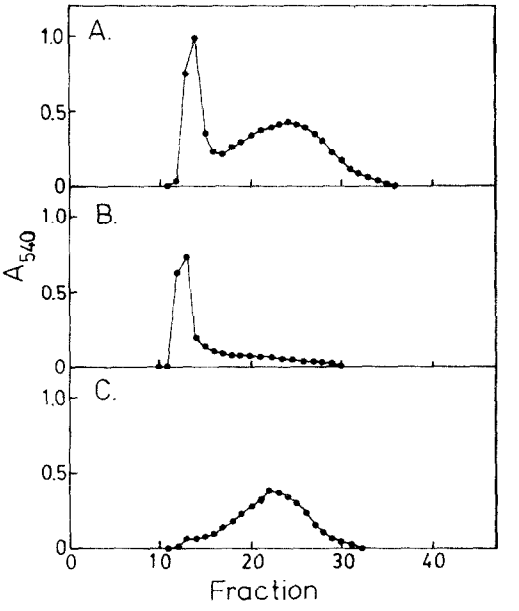

Fig. 2. Sepharose-CL-2B gel filtration of phytoglycogen from *sugary* endosperm and phytoglycogen fractions obtained after centrifugation at 14000 *g*. A, Phytoglycogen; B, 14000 *g* pellet; C, 14000 *g* supernatant.

starch from IS4577 seeds. This difference may reflect some out-crossing. High amylose content and variation between lines were also observed for the starches from different *su* lines of maize [7]. Therefore, the starch granule structure and composition of *su* sorghum are similar to *su* maize starch granules.

The water-soluble polysaccharide from *su* sorghum endosperm was compared to rabbit liver glycogen and maize phytoglycogen. The iodine absorption spectra, conversion to maltose by β-amylase, and average chain length were comparable for all three polysaccharides (Table 2). Chain lengths were similar when determined after isoamylase debranching or by periodate oxidation. All three glycogens were not debranched by pullulanase. These results suggest that all three glycogens are similar in structure. The phytoglycogen from *su* sorghum was further characterized by gel filtration (Fig. 2A). A bimodal distribution of polysaccharides was observed. Centrifugation of the phytoglycogen at 14000 g for 30 min was effective in separating the two classes of phytoglycogen (Figs. 2B and 2C). Chain lengths of 9.4 and 13.4 were found for polysaccharides in the 14000 *g* pellet and supernatant fractions, respectively. When maize phytoglycogen was fractionated by the same procedures, pellet and supernatant fractions had chain lengths of 8.3 and 12.2.

The distribution of unit chains of glycogens generated by enzymic debranching can be compared by gel filtration [13]. The gel filtration profiles of debranched rabbit liver glycogen, maize phytoglycogen and sorghum phytoglycogen are shown in Fig. 3. The profiles of all three glycogens showed broad distributions of chains with chain lengths ranging from 5 to 25. Peak fractions had chain lengths of 10–15. These results are in agreement with previous studies of glycogens and maize phytoglycogen [13].

The results of this study demonstrate the similarities of the starches and phytoglycogens from endosperms of *su* sorghum and *su* maize. The homology of the *su* mutants is not surprising considering that other classes of homologous starch mutants have been observed [11]. At present, no explanation for the formation of phytoglycogen in *su* maize is known at the enzymatic level. However, starch appears to be the precursor for phytoglycogen [14, 15] and the alteration of starch granule structure in *su*

Table 2. Properties of phytoglycogen isolated from *sugary* maize and *sugary* sorghum IS 4668 seeds and rabbit liver glycogen*

Polysaccharide	Chain length†	Conversion to maltose by β-amylase	I_2 spectra (λ_{max})
Sorghum phytoglycogen	12–16	49–65	470–480
Maize phytoglycogen	10–16	45–60	450–460
Rabbit liver glycogen	12–14	46–66	460–480

*Values are presented as ranges from typical determinations.

†Determined by enzymatic debranching and sodium iodate oxidation.

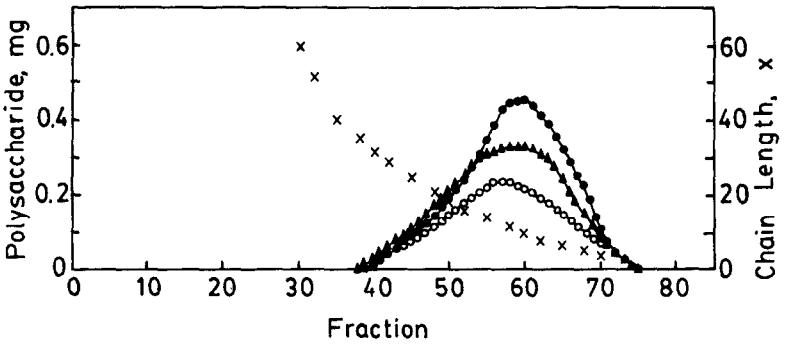

Fig. 3. Sephadex G-75 gel filtration of the products of isoamylase debranching of rabbit liver glycogen (○), maize phytoglycogen (●) and sorghum phytoglycogen (▲). Average chain length of unit chains found in a fraction (×).

endosperm was concluded to be necessary for the formation of phytoglycogen [14]. Further study of the fine structure of non-mutant and *su* starch granules and associated proteins are needed to describe more clearly the mutant effects. As the *su* mutants appear to affect the early stages of starch granule formation, the study of starch granules in these mutants should provide new insights into the basic processes of starch granule initiation and growth.

EXPERIMENTAL

Materials. Open-pollinated seeds from four sorghum accessions, reported to contain the *sugary* gene, were obtained from Dr. J. Axtell, Purdue University, West Lafayette, IN, U.S.A. Maize phytoglycogen was purified as part of a previous study [7]. Rabbit liver glycogen Type III and pullulanase were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. *Pseudomonas amylodermosa* isoamylase was purified from cultures as previously described [16]. All other reagents were of the highest purity available.

Extraction of starch granules and water-soluble polysaccharide. Seeds were soaked overnight in 0.02 M Na acetate, pH 6.5, 0.01 M $HgCl_2$. The following day pericarp and germ were removed and discarded. The endosperms were soaked an additional 24 hr, and then homogenized. Starch and water-soluble polysaccharides were extracted and dispersed as described earlier [11].

Characterization of glucans. I_2 spectra were determined with the I_2 reagent of Krisman [17] prepared with H_2O or saturated $CaCl_2$. Polysaccharides were fractionated by gel filtration on Sepharose-Cl-2B columns (1.5 × 40 cm) and fractions were assayed with I_2 reagent [11]. Average chain lengths of branched α-1,4-glucans were calculated from the ratio of total glucose equivalents to reducing equivalents after enzymatic debranching [18] and by $NaIO_4$ oxidation [18]. β-Amylase digestions were performed with standard reaction conditions [19]. Chains liberated by enzymatic debranching were separated by gel filtration on Sephadex G-75 columns (1.5 × 100 cm). Carbohydrate was detected in column fractions by the phenol–H_2SO_4 method [20] and the column calibrated as described previously [13].

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