CARBOHYDRATES

Dosage Effect at the Sugary-2 Locus on Maize Starch Structure and Function

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ABSTRACT

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Structural characteristics and functional properties of starches isolated from kernels of a sugary-2 (st2) dosage series were examined to determine whether the normal allele (St2) was completely dominant to the recessive allele (st2). Differential scanning calorimetry revealed intermediate values for gelatinization onset, gelatinization peak, range, total enthalpy, and retrogradation among genotypes possessing one and two mutant st2 alleles. No effect of gene dosage on amylose content was observed, but X-ray diffraction patterns revealed an intermediate degree of crystallinity relative to normal and mutant genotypes upon addition of two st2 alleles. Development of a peak at 19°C became more evident upon increasing doses of the st2 allele. Viscosity of the starch paste and gel strength resulting from the genotype possessing two doses of the st2 allele exceeded that of both mutant and normal genotypes. An intermediate retrogradation as detected by differential scanning calorimetry and from gel strengths of starches after storage for seven days suggested an increased stability of the St2St2st2st2 starch relative to that of normal starch.

The recessive sugary-2 (st2) allele of maize (Zea mays L.) was identified by Eyster (1934) and found to reside on chromosome six of maize. Until now, it has not been associated with any genetic lesion. Characteristics distinguishing st2 from the normal (nonmutant) genotype include reduced starch content, a 10–15% increase in amylose content, and a lowered birefringence end-point temperature, reflecting its low gelatinization temperature (Pfahler et al 1957, Kramer et al 1958). More recently, Inouchi et al (1984) found gelatinization endotherms from differential scanning calorimetry (DSC) to have lower onset (T0) and peak (Tp) temperatures and a reduced enthalpy (ΔH) when compared with normal corn starch. They also described starch of st2 as having an A-type X-ray diffraction pattern. However, the diffraction peaks were broad and weak, reflecting a lower degree of crystallinity than that of normal starch. Compared with other maize genotypes, a high susceptibility of st2 starch to digestion by pancreatic α-amylase was demonstrated by Sandstedt et al (1962). It was suggested that starch of st2 may differ from normal starch because of differences in the bonding of starch molecules or anomalous linkages within the molecules. Little difference in the fine structure of amylopectin from st2 was found as compared with normal starch on the basis of unit chain length distributions (Inouchi et al 1987). Takeda and Preiss (1993), however, found that, when compared with that of normal starch, the amylopectin of st2 starch was composed of larger sized long B-chains, which were more poorly branched, leading to increased iodine affinity value.

Interest in the st2 mutant emerged when starch isolated from genotypes containing this allele in combination with dull (dull) and sugary-1 (s1) was found to contain about 77% amylose (Dvovich et al 1951, Dunn et al 1953). Improved nutritional quality of st2 also was reported as a result of its high susceptibility to α-amylase digestion. This led Sandstedt et al (1962) to suggest its use in improving feed value. Enhanced grain quality was later recognized by Glover et al (1975) because the st2 allele in combination with opaque-2 (O2) resulted in kernel density nearly equal to that of ordinary ears. More recently, several novel starches resulting from st2 alone and in combination with other alleles such as d1, amylose-extender (ae), and waxy (wx) have produced starches with properties resembling those of some modified starches (Friedman et al 1988a, b; White et al 1993).

The lack of complete dominance of normal alleles at the mutant loci ae and wx on amylose content has been well documented (for review see Shannon and Garwood 1984). Similar studies have shown that gene dosage effects of st2 on amylose content do not exist, with the exception of its presence in a st4 background (Kramer and Whistler 1949). Studies considering only amylose content, however, may fail to reveal dosage effects on other starch characteristics. For example, Boyer et al (1980) found that, although increasing doses of ae in a wx background did not increase amylose content, average chain length did increase. Also, Yamada et al (1978) found that, upon addition of the ae allele in a wx background, gelatinization temperature increased.

Sanders et al (1990) emphasized the need for novel starches from mutant genotypes having the desired properties of chemically modified starches in order to avoid problems associated with regulatory approval for food use. Characterization of starches of less-used endosperm mutants, intermediate in expression, may reveal starch types with unique properties. The objective of this study was to investigate possible gene dosage effects at the st2 locus and determine whether novel starch properties exist among the dosage intermediates.

MATERIALS AND METHODS

Material

In 1992, self-pollinations and reciprocal crosses were made among stocks of Oh43 and Oh43 st2St2st2St2 in a nursery near Ames, IA. Mature ears were harvested and dried at 38°C for five days to ~13% moisture content. Samples were stored in a cold room at 4°C and 45% rh until analyzed.

Starch Isolation

A small-scale starch isolation procedure was used for the bulk of five kernels obtained from the center portion of the ear as described by White et al (1990). Ten separate extractions were made, each from a separate ear, from each genotype for DSC and colorimetric determination of amylose (AM) content. For all other analyses (iodine affinity, X-ray diffraction, amylography, and gel strength), starch was isolated from a single large-scale isolation by pooling the remaining grain of the same 10 ears. The large-scale starch isolation procedure used was that described by Steinke and Johnson (1991). After starch isolation, samples were purified with 5 volumes of 0.2 M sodium chloride-toluene (5:1, v/v) at least five times, and starch granules were allowed to settle. The final sediment was washed three times with distilled water and dried at 45°C for 24 hr. Because of the low gelatinization

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Amylose Determination

AM content was determined colorimetrically by dissolving ~5.0 mg of starch in 10 ml of 90% dimethyl sulfoxide containing 6 × 10⁻³ iodine (Knutson 1986). One milliliter of the dissolved sample was diluted to 9 ml with H₂O, and the absorbance was measured at 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Purified AM was prepared from maize starch as described by Schoch (1942) and used to construct a standard curve. Iodine affinity (IA) was determined by amperometric titration from starches of each genotype as described by Schoch (1964). These starches were defatted by refluxing 24 hr in 85% methanol. Values for IA were expressed as milligrams of iodine bound to 100 mg of starch.

Differential Scanning Calorimetry

For DSC analysis, a Perkin-Elmer DSC 7 analyzer equipped with a thermal-analysis data station (Perkin-Elmer Corp., Norwalk, CT) was used. Analysis of starch gelatinization was conducted as described by White et al. (1990). Approximately 4.0 mg (dwb) of starch was weighed into aluminum sample pans and 8 mg of distilled water was added. Samples were heated from 30 to 100°C at a rate of 10°C/min. DSC parameters for this study included enthalpy (ΔH), gelatinization onset temperature (Tₒ), peak temperature (Tₚ), and range. The parameters Tₒ, Tₚ, and ΔH were given directly by the DSC software. Range was calculated by (Tₚ − Tₒ) according to Krueger et al. (1987). Samples were stored for 7 days at 4°C and rerun to determine starch gel retrogradation (%R) as described by White et al. (1989).

Starch Granule Size

Isolated starch granules were dispersed in ethanol and mounted on slides. The preparations were placed on a Laborlux light microscope fitted with a television camera. The microscope system was attached to a digitizing Colorado video unit linked to a Kevex Delta IV (San Carlos, CA) computer with an image analysis software program. Fields of starch granules from different samples were viewed with a 40X objective and then digitized and processed for image analysis. Data collected were expressed in Waddell diameter and averaged from at least 200 granules per genotype.

X-Ray Diffractometry

X-ray diffraction patterns were collected with a Siemens (Madi- son, WI) D-500 X-ray diffractometer equipped with a copper X-ray tube and graphite monochromator. Samples containing 10% moisture were scanned from 4 to 40° 2θ. The degree of crystallinity was determined from a single diffraction pattern by using the method of Koksoy et al. (1993). Relative crystallinity (RC%) was calculated as the ratio of the upper crystalline area to the lower amorphous area of the diffraction pattern. The total area was calculated as the sum total of the crystalline and amorphous areas.

Amylography

A Brabender Viscoamylograph (C.W. Brabender Instruments, S. Hackensack, NJ) equipped with a 700-cmg sensitivity cartridge operating at a bowl speed of 75 rpm was used to determine pasting characteristics of starch suspensions (8% w/w, dwb) adjusted to a pH of 5.5. The temperature was raised from 30 to 95°C at a rate of 1.5°C/min and maintained at 95°C for 30 min, then lowered to 50°C at the same rate and held for 30 min. Measurements for each genotype were made in duplicate.

Gel Strength

Starch pastes from each genotype prepared by using the Brabender Viscoamylograph were used to measure gel strength after storage for one and seven days at 4°C. Starch paste was poured into aluminum dishes (27 mm i.d. × 27 mm), taped around the rims to increase the depth, and later cut back to produce a fresh surface before analysis. From each of two replicates, gel strength was measured at five different locations on each gel sample (five measurements per dish = total of four dishes) by using a Vomal texture analyzer (Texture Technologies, Scardan, NY) as previously described by Takahashi et al. (1989).

RESULTS AND DISCUSSION

AM Content

Results obtained from the two methods used for the determination of AM are shown in Table I. The AM% in starch of the two intermediate genotypes (St₁₂St₁₂St₁₂ and St₁₂St₁₂St₃) did not differ from the normal parent with respect to AM for either method of determination. The data are in agreement with previous studies, which concluded that gene dosage has no effect on amylose content.

Thermal Properties

Representative DSC thermograms of starch gelatinized from each genotype are shown in Figure 1. DSC parameter means of thermograms determined from 10 ears per genotype are given in Table II. Thermal properties, including Tₒ, Tₚ, and ΔH from starch of the homozygous recessive St₁₂ genotype, were lower than those of normal starch, which is in agreement with results obtained by Inouchi et al. (1991). The lowered Tₚ corresponds to the observed loss of birefringence occurring at a lower temperature in St₁₂ compared with that of normal starch (Pahler et al. 1957, Kramer et al. 1958). Brown et al. (1971) suggested that the lower gelatinization temperature caused by the St₁₂ allele may result from a reduction in the association between starch molecules within the granule. Renaturing the samples after storage at 4°C revealed that the %R of St₁₂St₁₂St₁₂ starch was substantially less than that of normal corn starch. The low %R reflects a possible increase in stability of the starch gel as discussed by White et al. (1989).

In contrast to the AM%, a dosage effect at the St₁₂ locus was present for Tₒ, Tₚ, range, and ΔH. A single dose of St₁₂ significantly reduced Tₒ and Tₚ, although the differences were not large and more closely resembled values from normal maize starch. Two doses of the St₁₂ allele resulted in Tₒ and Tₚ nearly intermediate to that of the normal and mutant genotypes. Also, the range increased to a value similar to that of the mutant, and ΔH was reduced relative to the normal genotype. The %R of the sample also decreased upon each addition of the mutant allele, indicating a possible increase in gel stability. Clearly, characteristics of the mutant genotype governing thermal properties were retained in the dosage intermediates, and these characteristics did not seem to be a result of increased AM.

| TABLE I
| Amylose Content (AM) and Average Granule Diameters of Starches from the Sugary-2 Dosage Series in the Maize Inbred Ob43 |
|-----------------|-----------------|-----------------|-----------------|
| Genotype        | AM, %           | Iodine Affinity | Average Granule |
| St₁₂St₁₂St₁₂    | 25.1            | 5.0             | 5.7 ± 1.5³     |
| St₁₂St₁₂St₃     | 24.7            | 4.9             | 6.0 ± 1.7³     |
| St₁₂St₁₂St₃     | 24.7            | 5.0             | 5.4 ± 2.0³     |
| St₁₂St₁₂St₃     | 39.3            | 8.1             | 5.6 ± 1.5³     |
| LSD³             | 1.5             |                 |                 |

³Mean of 10 ears per genotype.
³Mean of two determinations.
³Standard deviation.
³Least significant difference (P ≤ 0.05).
Starch Granule Size

Means for average granule diameters for genotypes are shown in Table I. Mean granule diameters were small compared to those previously reported for normal and su2 starch (Brown et al 1971). These differences may have been the result of the method chosen for analysis, of growing conditions, or the effect of genetic background. No substantial difference in mean diameter was seen among genotypes from the su2 dosage series. The results are in agreement with previous studies, indicating that starch granule sizes of the su2 mutant were similar to those of normal (Brown et al 1971). Differences in thermal properties of dosage intermediates compared to normal starch, therefore, did not seem to be related to size of granules.

X-Ray Diffraction

An A-type X-ray diffraction pattern was observed for all four genotypes (Fig. 2). Previous studies (Dvorch et al 1951, Inouchi et al 1984) also indicated an A-type pattern for starch of su2, with the exception of a study by Creech (1968) in which a B-type pattern was described. Diffraction peaks of su2,su2,su2 starch were more broad and weaker than were peaks of normal starch as a result of the decreased crystallinity of the starch. Inouchi et al (1984) noted that, because crystallinity is primarily caused by the amyllopectin fraction, su2,su2,su2 starch should be expected to have a lesser degree of crystallinity than that of normal starch. Genotypes with AM contents similar to each other, such as normal and the dosage intermediates, would be expected to have relatively similar crystallinities. X-ray diffraction patterns of dosage intermediates also revealed an A-type diffraction pattern resembling that of normal corn starch. RC% values among the genotypes shown in Table III suggest a dosage effect in the presence of two su2 alleles where RC was reduced from 46% seen in normal corn to 40%. Close examination of diffraction patterns suggest a slight increase in the peak at 19°2θ upon increasing doses of the su2 allele. This increase may be a result of the diminishing peak at 17°2θ allowing better resolution of the peak. Results of this study indicate that a structural component of the starch other than amylopectin may be involved in influencing relative crystallinity of the starch.

Pasting Properties

Values describing significant points of the amylogram are shown in Table IV. Starch of the su2,su2,su2 mutant clearly differed from

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**Table II**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tgo °C</th>
<th>Ttp °C</th>
<th>Range °C</th>
<th>ΔH, cal/g</th>
<th>%Rd</th>
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</thead>
<tbody>
<tr>
<td>Su2Su2Su2</td>
<td>67.3</td>
<td>71.0</td>
<td>7.4</td>
<td>2.9</td>
<td>58.8</td>
</tr>
<tr>
<td>Su2Su2su2</td>
<td>65.9</td>
<td>69.6</td>
<td>7.4</td>
<td>2.8</td>
<td>54.5</td>
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<tr>
<td>Su2su2su2</td>
<td>62.3</td>
<td>66.9</td>
<td>9.3</td>
<td>2.5</td>
<td>52.4</td>
</tr>
<tr>
<td>su2su2su2</td>
<td>58.3</td>
<td>63.0</td>
<td>9.4</td>
<td>0.9</td>
<td>43.9</td>
</tr>
<tr>
<td>LSD</td>
<td>0.8</td>
<td>0.5</td>
<td>1.0</td>
<td>0.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

a. Gelatinization onset.
b. Gelatinization peak.
c. Enthalpy of gelatinization.
d. Percent retrogradation; based on ΔH retrogradation/ΔH gelatinization.

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**Table III**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RC%</th>
<th>Total Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Su2Su2Su2</td>
<td>46.3</td>
<td>59.1</td>
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<tr>
<td>Su2Su2su2</td>
<td>46.4</td>
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<td>Su2su2su2</td>
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<td>59.8</td>
</tr>
<tr>
<td>su2su2su2</td>
<td>21.6</td>
<td>56.8</td>
</tr>
</tbody>
</table>

a. Relative crystallinity = upper crystalline area/lower amorphous area.
b. Sum total of crystalline and amorphous areas.
TABLE IV
Pasting Properties Determined from Brabender Viscoamylograph of Starches from the Sugary-2 Dosage Series in the Maize Inbred Oh43a

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pasting Onset (°C)</th>
<th>Peak Temperature (°C)</th>
<th>Peak Viscosity</th>
<th>95°C</th>
<th>95°C Hold</th>
<th>50°C</th>
<th>50°C Hold</th>
<th>Setbackb</th>
<th>50°C Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sst2St2St2</td>
<td>70</td>
<td>88.8</td>
<td>870</td>
<td>765</td>
<td>575</td>
<td>1,240</td>
<td>670</td>
<td>1,210</td>
<td></td>
</tr>
<tr>
<td>Sst2St2St2</td>
<td>68</td>
<td>89.8</td>
<td>870</td>
<td>810</td>
<td>620</td>
<td>1,360</td>
<td>740</td>
<td>1,415</td>
<td></td>
</tr>
<tr>
<td>Sst2St2St2</td>
<td>70</td>
<td>94.2</td>
<td>820</td>
<td>820</td>
<td>700</td>
<td>1,660</td>
<td>960</td>
<td>1,630</td>
<td></td>
</tr>
<tr>
<td>sst2sst2St2</td>
<td>92</td>
<td>...c</td>
<td>...c</td>
<td>20</td>
<td>60</td>
<td>320</td>
<td>260</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>LSDc</td>
<td>2.6</td>
<td></td>
<td>72</td>
<td>43</td>
<td>17</td>
<td>53</td>
<td>36</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

aStarch concentration of 8% (w/w, dwb). Values are means of two determinations.
bSetback denotes the difference between viscosity after a 30-min hold at 95°C and immediately after cooling to 50°C.
cNo peak observed.
dLeast significant difference (P ≤ 0.05).

TABLE V
Gel Strength of Starches from the Sugary-2 Dosage Series in the Maize Inbred Oh43a

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Firmness, g-force</th>
<th>Stickiness, g-force</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1b</td>
<td>D7c</td>
</tr>
<tr>
<td>Sst2St2St2</td>
<td>42</td>
<td>62</td>
</tr>
<tr>
<td>Sst2St2St2</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>Sst2St2St2</td>
<td>49</td>
<td>57</td>
</tr>
<tr>
<td>sst2sst2St2</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>LSDc</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

aValues are the average of two separate samples (five determinations per sample) from each of two replicates.
bSamples analyzed after one day of storage at 40°C.
cSamples analyzed after seven days of storage at 40°C.
dLeast significant difference (P ≤ 0.05).

that of normal corn starch by having a delayed pasting onset, no initial viscosity peak, and a low final viscosity after cooling. The pasting pattern of the Sst2St2St2 starch resembled that of other starch types, such as du1, which was reported by Wang et al (1992) to have a higher pasting onset temperature and a lower viscosity than that of normal starch. The relatively high AM content of du1 was thought to cause the higher pasting temperature resulting from the increased bonding of amylose molecules within the granule. A more loosely branched amylpectin structure of du1 ae relative to other high-amylose starches was suggested by Katz (1991) to result in a pasting property similar to that of du and sst2. The sst2 starch also may have a loosely branched amylpectin structure, thus accounting for its unusual pasting properties. This idea is in agreement with observations of Brown et al (1971) describing a reduced association of molecules in the granules of sst2. Effects of gene dosage on pasting properties were observed (Table IV). An increased peak temperature occurred with each addition of the sst2 allele, and two doses significantly (P < 0.05) increased the temperature from 88.8 to 94.2°C. Pasting onset temperature measured for values exceeding 10 BU did not differ greatly among dosage intermediates and normal starch, but starch possessing two alleles of sst2 lagged in viscosity after pasting onset when compared with normal starch. Pasting onset temperature of the genotype possessing two sst2 alleles, however, was intermediate relative to the normal and mutant genotype when based on values exceeding 100 BU. Viscosities of pastes from dosage intermediates upon heating to 95°C and until completion of the cooling cycle exceeded those of both normal and mutant genotypes. A structural basis for the increased viscosity of the dosage intermediates relative to normal starch is unclear. Characteristics of the fully recessive sst2 starch (other than AM content) are likely to be retained in the intermediates and to play a role in starch structure. Structural changes leading to the reduced relative crystallinity in the Sst2St2St2 genotype are examples. The double mutant combination of ae wx described by Yamada et al (1978) also possessed pasting properties similar to that of the Sst2St2St2 genotype with respect to the higher pasting onset temperature; it maintained a more viscous paste than did both normal and waxy. Structurally, ae wx starch differs from waxy and normal starches in having longer mean chain lengths. Further studies may reveal unit chain length differences between normal starch and starches of the sst2 dosage intermediate genotypes.

Gel Strength
Table V shows gel strengths of starch pastes prepared in the Brabender Viscoamylograph after storage for one and seven days. Gels from normal starch were almost twice as firm as those from Sst2St2St2 starch after one day of storage. Also, the addition of sst2 alleles increased starch gel strength to values greater than that of normal starch. These results correspond to final viscosities of starch pastes as determined by amylography. Although, no structural basis for the differences in gel strength is clear, a more concentrated soluble phase or a more linear amylose of dosage intermediates may result in the increases (Takahashi and Seib 1988). After seven days of storage, gel strength increased for all genotypes. Gel strength of sst2St2St2 samples remained weakest; no significant differences were seen among the normal and dosage intermediate genotypes. Interestingly, the ratio of firmness of gels between one and seven days of storage was lower for all genotypes containing at least one sst2 allele. Because retrogradation of starch gels is associated with syneresis of water, leading to denser gels, the change in firmness may reflect stability of gels under refrigeration storage, with the greater the relative increase in firmness during storage, the greater the tendency to retrograde. These findings agree with the DSC results in which there was a decrease in degree of retrogradation of starches with each addition of a sst2 allele. Gel stickiness after one day of storage revealed that the value for Sst2St2St2 was greater than that of normal starch, whereas genotypes containing one or two sst2 alleles were intermediate. After seven days, however, no significant differences were seen. A continuous decrease in the ratios of values determined after one and seven days was seen upon each addition of the sst2 allele.

CONCLUSIONS

The sst2 mutant of maize was completely recessive to the normal allele with respect to AM content, thus confirming previous findings. Thermal properties measured by DSC clearly revealed a dosage effect for all parameters. Certain structural characteristics of starch from the Sst2St2St2 genotype seemed to be retained in the dosage intermediates, because relative crystallinity decreased in starch of the Sst2St2St2 genotype. Functional properties of starches from the Sst2St2St2 genotype also differed from those of normal and mutant genotypes because pasting onset was delayed, and final viscosity was higher than in normal starch. Gel strengths also exceeded that of the normal genotypes.

This study has shown that novel starch types can be achieved through development of dosage intermediate genotypes. Commercial production of starch from dosage intermediates, however, would not be practical because of the segregating nature of kernels in hybrid corn. Starch from hybrid sst2St2St2 genotypes with traits of the dosage intermediates may be possible through the use of
modifying genes or the development of additional alleles at the su2 locus (Curme 1955; Vineyard et al. 1958).

LITERATURE CITED


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