Thermal Behavior During Gelatinization and Amylopectin Fine Structure for Selected Maize Genotypes as Expressed in Four Inbred Lines[1]

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ABSTRACT

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To evaluate the effect of mutant genotype on starch gelatinization behavior, a series of starches in the Ia5125 inbred line was studied by differential scanning calorimetry (DSC). To examine the effect of amylopectin structure on gelatinization behavior, those maize samples containing the *wx* gene were also studied by size exclusion high-performance liquid chromatography (HPLC) after isoamylase debranching. Thermal analysis of starch gelatinization by DSC and the pattern of debranched chains by HPLC were then observed for four mutant genotypes (*wx*, *ae wx*, *du wx*, and *ae du wx*) from four inbred lines (Ia5125, Ia453, S3-61, and W64A). Within a line the DSC maximum temperature (T_{max}) for the *ae wx* genotype ranged from 5.1 to 14.3°C above that for the genotype with the next highest T_{max}. Enthalpy was highest for the *ae wx* starches in the S3-61 and W64A lines. The generally bimodal HPLC chromatograms had a greater proportion of the total area in the high molecular weight peak for the *ae wx* starches. Furthermore, the higher molecular weight shoulder of the low molecular weight peak became the dominant low molecular weight peak for the *ae wx* starches from the S3-61 and W64A lines. Either or both of these differences in amylopectin fine structure could explain the different thermal behavior of the *ae wx* starches during gelatinization. It appears that variation in the proportion of chains between and within the two major peaks, rather than displacement of the entire chain length distribution, may be responsible for variation in amylopectin structure. Variation in these proportions may help explain differences in physical properties of the starches.

The physical behavior of dent maize starch is unsuitable for many industrial applications. To meet the needs of starch users, chemical modifications (cross-linking or substitution, or both) and physical modifications (pregelatinization) are often made. Another strategy is the use of starch from mutant maize genotypes. To date, waxy maize starch (from the *wx* genotype) is of greatest commercial importance, followed by high-amylose maize starch (from the *ae* genotype). The combination of modification and the use of starch from a mutant genotype is presently of great importance in production of specialty starches, as indicated by the great variety of modified waxy starches available. Innovative chemical modification techniques face severe hurdles in achieving regulatory approval; thus a greater potential for manufacture of modified starches with improved functionalities lies in use of different raw materials. For maize starch, the effect of inbred line and of various available mutant genotypes on properties of the resulting starch has not been examined in depth. These starches could be important to the food industry in two ways: in unmodified

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form, they might have properties that would allow a manufacturer to use them and avoid the term "modified starch" on the label, and they would provide the raw material for modification to provide starch with improved physical behavior.

The best-known effect of the mutant maize genotypes is on the proportion of amylose and amylopectin: high-amylose maize starches have 50–70% apparent amylose, whereas waxy maize starch has essentially no amylose (Shannon and Garwood 1984). What is less commonly appreciated is that the structure of the amylopectin is also affected in the mutant genotypes. In fact, the apparent amylose values for high-amylose starch are probably not good estimates of the actual amylose content because iodine binding by the amylopectin in high-amylose starches contributes to the apparent amylose values (Boyer and Liu 1985). It is not clear how much the altered physical behavior of high-amylose starch is due to the proportion of amylose and how much is due to the altered amylopectin structure.

Average chain lengths of amylopectins from various plant species have been known to differ for some time (Lii and Lineback 1977, Whistler and Daniel 1984), but the specific nature of the branched structure has proved a formidable problem. The elucidation of amylopectin fine structure has evolved as analytical techniques have improved. The Haworth (Haworth et al 1937), Staudinger (Staudinger and Huseman 1937), and Meyer (Meyer and Bernfeld 1940) models were based on the observed proportion of 1-4 and 1-6 glycosidic linkages. Peat introduced the A, B, and C terminology for the types of linear chains (Peat et al 1952). The Meyer model was refined by Nikuni (1969) and French (1972) to a cluster model, which was further refined by Robin et al (1974). Recent improvements in the resolution of size exclusion high-performance liquid chromatography (HPLC) have led to a refinement of the model based on interpretation of the distribution of linear chains following isoamylase digestion (Hizukuri 1986, Inouchi et al 1987). In Hizukuri's model (1986) the B chains are considered according to how many clusters they span: B1 chains are localized to one cluster, B2 chains span two clusters, B3 chains span three clusters, and so forth. A chains are tacitly assumed to be localized to one cluster.

The important physical properties of starch materials include the thermal requirements for gelatinization, the viscosity generated by gelatinization, stability of gelatinized granules to continued heat and shearing action, and susceptibility of gelatinized starch to retrogradation. The physical behavior most readily studied on a small scale is the thermal behavior during gelatinization. The temperature of gelatinization may be studied by differential scanning calorimetry (DSC) or by loss of birefringence under a polarized light microscope equipped with a hot stage (Lund 1984). DSC has the advantage that in addition to the gelatinization temperature, the enthalpy of gelatinization may also be obtained. Donovan (1979) has described the theoretical interpretation of DSC data for starch gelatinization.

From the Pennsylvania State University maize breeding collection we have available starches from the endosperm of several mutant genotypes as expressed in four inbred lines (three sweet lines [Ia5125, Ia453, and S3-61] and a dent line [W64A]). One objective of the present work was to screen a selection of these samples for unique physical properties as observed by DSC.

Among the available mutant genotypes are several containing the *wx* gene, which is thought to result in a lack of amylose (Shannon and Garwood 1984). Assuming that the amylopectin fine structure of these samples differs, DSC analysis of these samples should provide insight into the magnitude of the potential contribution of amylopectin to granule physical behavior. A second objective was to examine the variation in thermal behavior of starches comprised of only amylopectin. A third objective was to begin the elucidation of the amylopectin fine structure of these samples to investigate the hypothesis that the physical behavior observed by DSC is related to some aspect of the amylopectin fine structure. For the second and third objectives, four *wx*-containing genotypes (*wx, ae wx, du wx*, and *ae du wx*) were examined as expressed in the four inbred lines.

MATERIALS AND METHODS

Starch Samples

Backcross conversions for the endosperm mutants waxy (*wx*), amylose-extender (*ae*), sugary (*su*), and dull (*du*) were produced by standard procedures in the maize genetics program at the Pennsylvania State University. Multiple mutant combinations were produced by crossing the backcross conversions of the single mutants, selfing, and selection for multiple mutant lines. All genotypes were verified by appropriate allele tests. Genotypes will be identified by those gene loci homozygous for recessive mutant alleles. Other gene loci not listed are therefore homozygous for the dominant allele.

Mature kernels (20) were soaked in 50 ml of a 20 m*M* sodium acetate, pH 6.5, 10 m*M* mercuric chloride solution. After 24 hr, the germ and pericarp were removed and 50 ml of fresh solution was added for an additional 24 hr (Boyer et al 1976). Endosperm was repeatedly homogenized for 1-min intervals in a mortar and pestle, and freed starch granules were collected by washing through a 44-μm mesh. Starch granules were purified by multiple extractions (8–12) with saline and toluene (Boyer et al 1976). Granular starch was washed three times with double distilled water, once with acetone, and dried at 40°C.

Isoamylase

Isoamylase was isolated from *Pseudomonas amyloderamosa* by the procedure of Yokobayashi et al (1970). No contaminating amylase activities were observed.

Thermal Analysis by Differential Scanning Calorimetry

Thermal analysis was performed using a differential scanning calorimeter (model DSC-4, Perkin-Elmer, Norwalk, CT). Starch powder samples of 1.5 mg were placed in preweighed, coated aluminum hermetic pans (no. 90001-201, DuPont, Wilmington, DE), and the precise weight was determined (Autobalance, Perkin-Elmer). Deionized water (15 μl) was added and the pan was sealed. The reference pan contained 15 μl of water. The heating rate was 10°C/min, from 15 to 115°C. Temperature and enthalpy were calibrated using indium. Endotherms were analyzed (Thermal Analysis Data Station, Perkin-Elmer) to calculate peak onset temperature (T_o), peak maximum temperature (T_{max}), and peak enthalpy (ΔH). Enthalpies were calculated on a starch weight basis. DSC data for five starch samples (from Ia5125 normal, *wx*, *ae*, *du*, and *ae wx*) were reported previously (Brockett et al 1988) based on estimation of starch content by total carbohydrate analysis. For all data reported in the present work, it was assumed that samples were 90% starch.

Amylopectin Fine Structure Analysis

Isoamylase digestion. Starch samples were dispersed in 90:10 (v/v) dimethyl sulfoxide/deionized water (hereafter termed 90% DMSO) by heating 10 min in a boiling water bath, cooling to room temperature, and heating again for 10 min. Dispersions were 10 mg/ml, and aliquots of 0.5–1.0 ml were subsequently digested. Aliquots were precipitated with three volumes of 1% KCl in 75% methanol, and centrifuged 5 min at 900 × *g*. The supernatant was discarded and the precipitate was resuspended in the original volume of 90% DMSO by heating in boiling water for 10 min. The methanol precipitation was repeated, and the precipitate was taken up in 1.8 times the original volume of 0.5*M* sodium acetate buffer at pH 3.5. An aliquot of isoamylase (1 mg/ml in 0.5*M* sodium acetate buffer at pH 3.5) was added to bring the total volume to two times the original volume. Digestion was performed at 35°C. After one week, additional isoamylase solution was added. Digestion was monitored by size-exclusion chromatography. Digestion was considered complete when the areas of the two major chromatographic peaks did not change by more than 2% in 24 hr.

Size-exclusion chromatography of enzyme digests. The methodology was adapted from that of Kobayashi et al (1986). The HPLC system consisted of a pump (model 510, Waters, Milford, MA), an injector (U6K, Waters), a 2-cm guard column

(LC-18 Pelliguard, Supelco, Bellefonte, PA), two 30-cm size exclusion columns (Zorbax PSM 60S, DuPont, Wilmington, DE) in series, a differential refractometer (model 410), and an integrator (model 745, Waters). The mobile phase was DMSO and the flow rate was 0.4 ml/min. The columns and detector were maintained at 40°C.

A 0.050-ml aliquot was removed from the isoamylase digestion mixture and added to 0.450 ml of DMSO. The mixture was heated in boiling water for 10 min in a capped 1.5-ml tube, and then centrifuged for 5 min at 2,500 × *g*. A 100-μl portion of the clear supernatant was injected into the HPLC system.

Chromatograms were interpreted as having two major peaks separated by the minimum identified by the integrator, as indicated in the figures.

Estimation of chain lengths of debranched material. A sample of partially acid-hydrolyzed starch containing a range of chain lengths was made to 5 mg/ml in 90% DMSO. A 20-μl aliquot was injected into the HPLC system. The retention times for degree of polymerization (DP) 1–5 could be determined directly from the chromatogram.

This partially hydrolyzed starch was also made up in 0.01*N* NaOH (5 mg/ml), and 2 ml was applied to a size-exclusion column (Sephadex G-75, 1.5 × 100 cm). The mobile phase was 0.01*N* NaOH and the flow rate was 0.6–0.9 ml/min. Eighty 2.8-ml fractions were collected and analyzed for total carbohydrate by the phenol-sulfuric acid test (Hodge and Hofreiter 1962) and the Park Johnson test for reducing ends (Porro et al 1981). Based on these two analyses, the average chain length for each fraction was calculated. To calibrate the Sephadex G-75 column, linear regression was done on a plot of the log chain length versus fraction number. Aliquots (2 ml of 5 mg/ml) from two debranched starch samples were also applied to the column, and for each sample the DP of the two peaks was calculated. These peaks were assumed to correspond to the two peaks observed by HPLC for each sample.

A sample of commercially prepared potato amylose (no. A0512, Sigma Chemical Co., St. Louis, MO) was analyzed for total carbohydrate and reducing ends as above; the DP was calculated to be 330. This sample was made to 20 mg/ml of 90% DMSO and injected into the HPLC system. This retention time was combined with the retention times for DP 1–5 and for the debranched amylopectin samples above. The relationship between the natural logarithm of the degree of polymerization and retention time was fitted to a linear equation ($y = 12.6673 - 0.4397x$, $r = 0.98$), which was used to estimate the degree of polymerization of the observed HPLC peaks.

Statistics

Analysis of variance was done on the DSC data and on the HPLC data. Peak onset temperatures were not compared statistically due to the variable nature of this value when the endotherm was broad. Multiple comparisons were done by least significant difference (LSD) after a preliminary *F* test. Three-way analysis of variance was performed on the data for the four *wx*-containing genotypes from the four inbred lines. In this 2 × 2 × 4 factorial design, the two gene factors were the absence or presence of either *du* or *ae*; the third factor was the inbred line. Correlation analysis was performed on the means of HPLC chromatogram data and of DSC thermogram data.

RESULTS AND DISCUSSION

The Ia5125 Inbred Line

Thermal analysis. For the Ia5125 inbred line, the thermal behavior during gelatinization in excess water was studied for the normal starch and the single, double, and triple mutants based on the *wx, ae, du,* and *su* genotypes. The data are presented in Table I and thermograms are presented in Figure 1. For the single mutants, the T_{max} was highest for the *ae* starch at 77.6°C. Previous work with dent maize starch showed that T_{max} for the *ae* starch was higher than for other single mutants, but the temperature was much higher (Zobel 1984) than for this genotype in the Ia5125 line in the present work. In addition, the endotherm was much broader in the dent lines previously studied (Zobel 1984, Wooten and Bamunuarachchi 1979). The difference is at least partially due to line differences but may also involve annealing of those commercially steeped dent starches (Krueger et al 1987). Based on the peak height index criterion of Krueger et al (1987), starches in the present work were not annealed (Brockett et al 1988). Enthalpy values for all single mutant starches in the present work were about 3 cal/g, slightly higher than observed for the normal.

Starches from four of the six double mutant genotypes had similar T_{max} values to that of the normal starch. However, the *ae wx* genotype gelatinized at a higher temperature. This result might suggest that the *ae* genotype controls the gelatinization temperature; however, no elevated gelatinization temperature was observed for the *ae du* or the *ae su* genotypes. Furthermore, the double mutant with by far the highest T_{max} was *du su* at 94.6°C. Enthalpy values varied considerably for the double mutants; again the enthalpy of gelatinization for the *du su* starch was far higher than the others, at 4.7 cal/g. Although it appears plausible that the gelatinization temperature and enthalpy would be related, support for this relationship in Table I is equivocal at best.

The thermal behavior of the triple mutant starches cannot be predicted from that of the double and single mutants. The lowest T_{max} for any of the starches (64.3°C) was for the *ae du su*, despite the observation that the *du su* combination showed the highest T_{max} and the presence of the *ae* gene that also was responsible for a relatively high T_{max}. Again, the highest enthalpy and T_{max} were observed for the same sample, *ae su wx*.

It is instructive to observe the DSC thermograms for these starches (Fig. 1). The peak is relatively sharp and well defined for the normal and for *wx* and *du* starches. The peak is much broader for the *ae* starch. The thermogram for the *su* starch is a hybrid of the two types of peak: a sharp peak at the low temperature and a low broad peak at a higher temperature. For the double mutants, the second peak was observed at the higher temperature for two starches: *ae du* and *du su*. For the latter,

TABLE I
Thermal Analysis of Gelatinization of Starches from Several Mutant Genotypes in the Ia5125 Inbred Line[a]

Starch[b]	T_o (°C)	T_{max}(°C)	ΔH (cal/g)
Normal (3)	64.2 ± 0.2	69.4 ± 0.1 fg	2.7 ± 0.1 i
Single mutants			
wx (4)[c]	61.0 ± 0.1	69.8 ± 0.2 ef	3.4 ± 0.1 ef
ae (7)	68.8 ± 0.5	77.6 ± 0.8 b	3.1 ± 0.2 g
du (4)	61.0 ± 0.5	68.5 ± 0.2 h	2.9 ± 0.0 h[d]
su (3)	61.3 ± 0.2	68.3 ± 0.2 h	3.3 ± 0.0 f
Double mutants			
ae wx (4)[c]	69.8 ± 0.4	78.4 ± 0.2 b	3.5 ± 0.1 d
du wx (3)[c]	64.4 ± 0.8	71.5 ± 0.0 c	3.7 ± 0.0 c
su wx (3)	64.6 ± 0.6	71.0 ± 0.6 cd	3.9 ± 0.0 c
ae du (3)	59.9 ± 0.3	69.1 ± 0.9 fgh	2.4 ± 0.0 j
ae su (3)	55.7 ± 1.2	70.6 ± 1.0 de	3.5 ± 0.1 de
du su (3)	53.6 ± 0.6	94.6 ± 0.1 a	4.7 ± 0.2 a
Triple mutants			
ae du wx (3)[c]	54.8 ± 1.3	71.5 ± 0.7 cd	3.2 ± 0.0 f
ae su wx (3)	70.8 ± 0.2	78.1 ± 0.2 b	4.3 ± 0.2 b
du su wx (4)	60.9 ± 0.5	68.9 ± 0.1 gh	2.7 ± 0.1 i
ae du su (3)	51.9 ± 0.3	64.3 ± 1.2 i	3.1 ± 0.1 g

[a] Values are mean ± SD. In the same column, values followed by a common letter are not significantly different at $P < 0.05$, by LSD analysis. Pooled standard errors are not reported due to unequal *n*. Experimental error mean square for $T_{max} = 0.32$; for $\Delta H = 0.011$.
[b] Genotype (number of replicate analyses).
[c] Data for this sample are also found in Table III.
[d] Enthalpy for this sample was previously reported (Brockett et al 1988) based only on the main endothermic peak. The value here is slightly higher due to inclusion of the area of the secondary peak, as was done in the present work for other starches when a secondary peak was observed (Figs. 1 and 3).

this high-temperature peak was the dominant peak. For the triple mutants, only the *ae du su* starch showed even a hint of the high-temperature peak. It is of interest that only those starches lacking the *wx* gene showed the higher temperature peak. Both the *ae du su* starch and the *ae du wx* gave a very broad gelatinization endotherm. The *ae su wx* starch showed a strong low-temperature shoulder to the main gelatinization peak.

Amylopectin fine structure. Isoamylase digestions of the Ia5125 starches were only done for those starches containing the *wx* gene. The data are presented in Table II, and representative HPLC chromatograms are presented in Figure 2A. Each of the two major peaks was apparently made up of more than one component. By the model of Hizukuri (1986) the low molecular weight peak would be composed of A chains (slightly shorter) and B1 chains (slightly longer). The high molecular weight peak would be composed of B2, B3, and longer chains. Variation was observed in the proportion of the total area in the two major peaks and in the relative proportion of the components within each major peak. The latter was difficult to quantify but could be observed qualitatively. For example, the debranched *ae wx* starch differed from the *wx* starch in two respects (Fig. 2A): the proportion of material in the high molecular weight peak was greater, and the higher molecular weight component of the low molecular weight peak was more pronounced.

In all but two starches only the lower molecular weight component of the low molecular weight peak was consistently

TABLE II
Distribution of Linear Chains After Isoamylase Debranching and Size-Exclusion High-Performance Liquid Chromatography Analysis for *wx*-Containing Starches in the Ia5125 line[a]

Starch[b]	High-MW[c] Peak: Retention Time (min)	High-MW[c] Peak: Percent of Total Area	Retention Time (min): Main Low-MW Peak	Retention Time (min): Secondary Low-MW Peak
wx[d]	19.7±0.1 d	41±1 d	23.5±0.1 b	none[e]
ae wx[d]	20.0±0.2 bcd	50±1 b	23.3±0.1 c	22.4±0.0
du wx[d]	19.8±0.3 cd	38±1 e	23.7±0.2 ab	none[e]
su wx	21.0±0.3 a	48±0 b	23.7±0.0 a	none[e]
ae du wx[d]	20.3±0.3 bc	45±0 c	23.7±0.1 ab	22.2±0.1
ae su wx	20.5±0.3 b	56±1 a	23.3±0.1 c	none[f]
du su wx	20.4±0.4 b	37±2 e	23.8±0.1 a	none[e]

[a]Values are mean ± SD. In the same column, values followed by a common letter are not significantly different at $P < 0.05$, by LSD analysis. Pooled standard errors: high-MW retention time, 0.027; high-MW percent of total area, 0.346; low-MW retention time, 0.003.
[b]Genotype. Three replicate analyses for each genotype.
[c]MW = Molecular weight.
[d]Data also contained in Table IV.
[e]No peak was identified by the integrator, but a shoulder was present in this region.
[f]A peak was identified on two of the three chromatograms; retention times were 22.3 min.

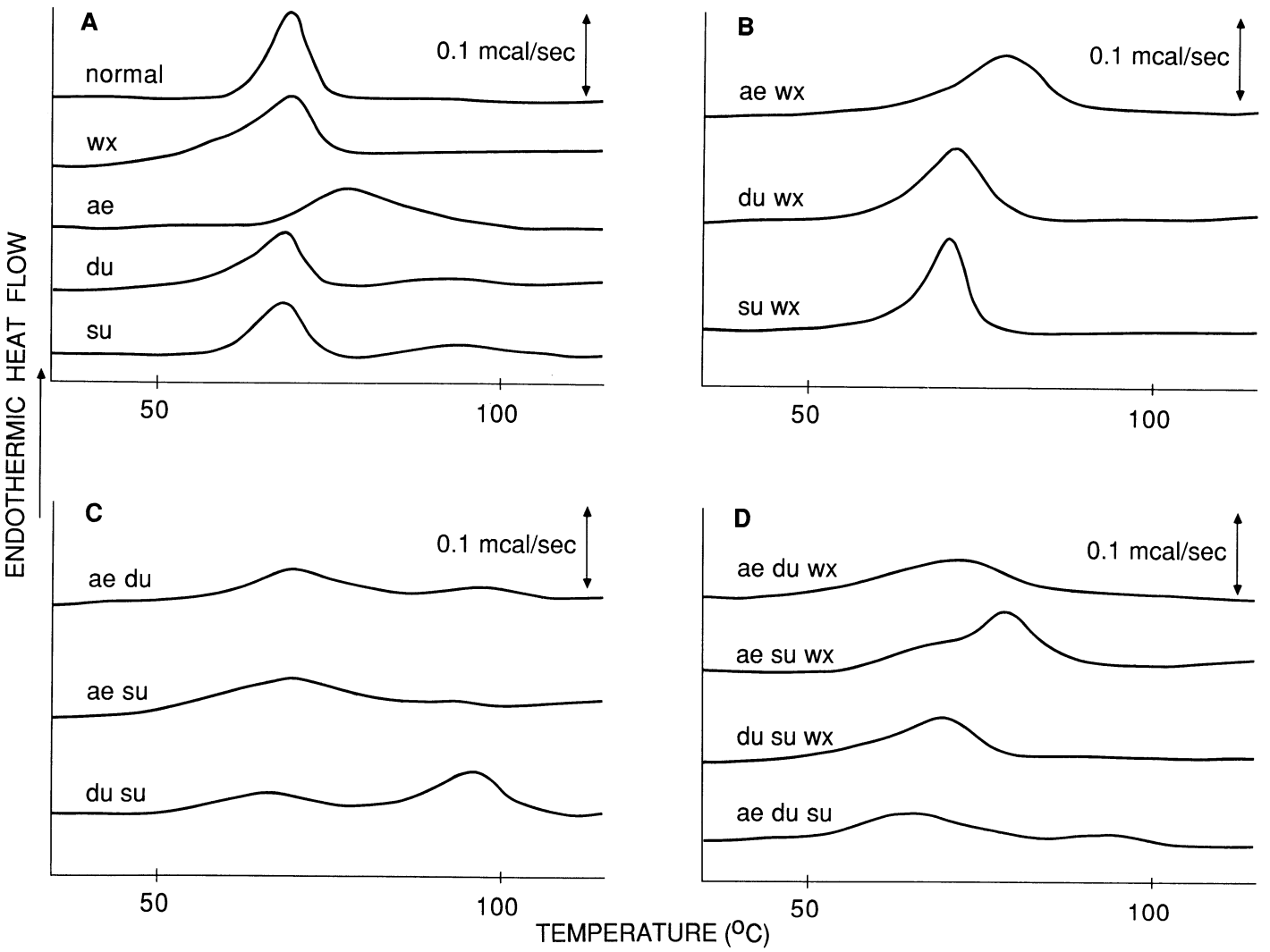

Fig. 1. Differential scanning calorimetry thermograms for selected mutant maize genotypes in the Ia5125 inbred line: single mutants (**A**), *wx*-containing double mutants (**B**), other double mutants (**C**), triple mutants (**D**).

detected by the integrator as a peak (Table II), although it is evident in Figure 2A that the higher molecular weight component of the low molecular weight peak was present in all samples. For the *ae wx* and *ae du wx* starches, a secondary low molecular weight peak was also consistently detected (Table II). Of the Ia5125 starches analyzed by both DSC and HPLC, it is only for the *ae wx* and the *ae su wx* starches that a higher T_{max} was observed. These two starches also had the highest proportion

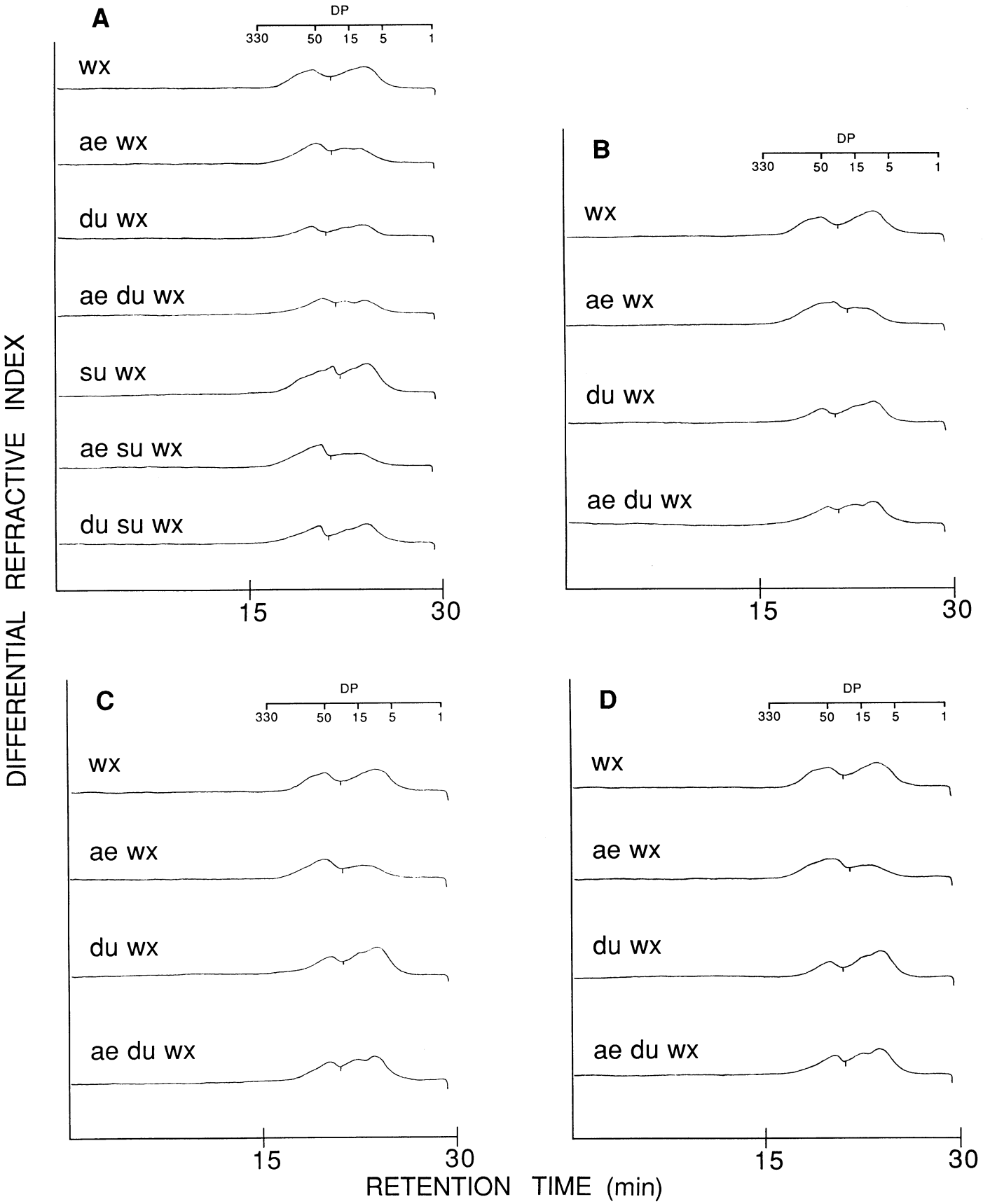

Fig. 2. Size-exclusion chromatograms for isoamylase-debranched starch from selected maize mutant genotypes as expressed in four inbred lines: Ia5125 (**A**), Ia453 (**B**), S3-61 (**C**), and W64A (**D**).

of the total material in the high molecular weight peak. The *su wx* starch also had a relatively high proportion of material in the high molecular weight peak, but for this starch the lower molecular weight component of the low molecular weight peak was proportionately large. Although the *ae du wx* starch does have a detectable secondary low molecular weight peak, the T_{max} is not high. However, for this starch the percent of the total area in the high molecular weight peak is relatively lower than for the *ae wx* and *ae su wx* starches. It is plausible to suppose that the proportion of two linear components within the low molecular weight peak, as well as the overall percent of the branches in the high molecular weight peak, may be important in determining the gelatinization T_{max} in this sweet line.

Starches from Four *wx*-Containing Genotypes in the Four Lines

Thermal analysis. DSC data for thermograms for gelatinization of starches from the *wx, ae wx, du wx*, and *ae du wx* genotypes is summarized in Table III. Representative thermograms are shown in Figure 3. T_{max} for these starches ranged from 64.4°C for the *wx* in Ia453 to 85.3°C for the *ae wx* in W64A. Within each line the highest T_{max} was for the starch from the *ae wx* genotype; two of these were above 80°C (80.5 and 85.3°C for the S3-61 and W64A, respectively). For the 16 starches, enthalpies ranged from 3.0 cal/g for the *wx* starch in W64A to 5.6 cal/g for *ae wx* in W64A. In three lines, the highest enthalpy was for starch from the *ae wx* genotype. As with T_{max}, the two highest enthalpies were for *ae wx* in the S3-61 and W64A lines (4.9 and 5.6 cal/g, respectively).

The shapes of the thermograms varied among the 16 *wx*-containing starches (Fig. 3). Within each line, the *wx* and *du wx* starches produced the sharpest peaks. The peak for the *ae du wx* starch was usually the broadest. The peak for the *ae wx* starch was intermediate. The thermogram for the *ae wx* starch from the W64A line apparently has two components.

The differences in thermal behavior seen among these 16 starches illustrate the complex role that amylopectin can play in determining gelatinization behavior (Table III). In all four lines, thermograms for the *ae wx* and *ae du wx* starches show relatively broad peaks. These broad peaks may be due to either a lack of cooperativity among crystallites within similar granules, to variation among granules, or both. Some of the broad thermograms appear to have two components, which might be interpreted as two overlapping peaks. Such an interpretation is consistent with the possibility that two populations of granule are present.

TABLE III
Thermal Analysis of Gelatinization of Starches from *wx*-Containing Genotypes from Four Inbred Lines[a]

Starch[b]	T_o (°C)	T_{max}(°C)	ΔH (cal/g)
Ia5125			
wx (4)	61.0 ± 0.1	69.8 ± 0.2 h	3.4 ± 0.1 gh
ae wx (4)	69.8 ± 0.4	78.4 ± 0.2 c	3.5 ± 0.1 f
du wx (3)	64.4 ± 0.8	71.5 ± 0.0 ef	3.7 ± 0.0 e
ae du wx (3)	54.8 ± 1.3	71.5 ± 0.7 ef	3.2 ± 0.0 hi
Ia453			
wx (4)	56.0 ± 0.5	64.4 ± 0.2 j	3.1 ± 0.1 ij
ae wx (3)	69.0 ± 0.7	77.4 ± 0.7 c	3.9 ± 0.0 cd
du wx (3)	64.1 ± 0.3	71.2 ± 0.0 efg	3.5 ± 0.0 fg
ae du wx (3)	58.8 ± 5.8	72.3 ± 0.3 e	3.1 ± 0.1 ij
S3-61			
wx (4)	60.1 ± 1.2	67.4 ± 0.2 i	3.3 ± 0.1 h
ae wx (3)	71.0 ± 1.1	80.5 ± 0.5 b	4.8 ± 0.2 b
du wx (3)	59.5 ± 0.8	70.2 ± 0.7 gh	3.8 ± 0.1 de
ae du wx (3)	54.2 ± 0.7	74.8 ± 0.9 d	3.1 ± 0.1 j
W64A			
wx (4)	66.7 ± 1.0	71.0 ± 0.8 fg	3.0 ± 0.1 j
ae wx (3)	66.9 ± 0.9	85.3 ± 1.2 a	5.6 ± 0.0 a
du wx (3)	60.9 ± 0.3	71.0 ± 0.4 fg	4.0 ± 0.0 c
ae du wx (3)	53.6 ± 0.1	69.4 ± 0.3 h	3.6 ± 0.1 f

[a]Values are mean ± SD. In the same column, values followed by a common letter are not significantly different at $P < 0.05$, by LSD analysis. Pooled standard errors not reported due to unequal *n*. Experimental error mean square for T_{max} = 0.308; for ΔH = 0.008.
[b]Inbred line and genotype (number of replicate analyses).

By 2 × 2 × 4 factorial analysis, the two gene treatments (±*ae* and ±*du*) the four lines, and the interaction terms were all highly significant ($P < 0.0001$) with respect to T_{max} and enthalpy. Consequently, both genotype and line influence thermal behavior during gelatinization, but the influence of a particular gene varies according to the presence of other mutant genes and according to the line. Thus with respect to gelatinization, the influence of genotype and line is highly complex and deserves further study.

Amylopectin fine structure. HPLC chromatograms of debranched starches from all four lines are presented in Figure 2. The retention times for the two major peaks and the percent of the total area in the high molecular weight peak are presented in Table IV. For the 16 samples, retention times for the two peaks were generally similar, although significant differences were observed. The mean retention time for the high molecular weight peak for all 16 starches was 20.0 min, which corresponds to a DP of 48. The individual retention times corresponded to a range of DP 42–58 (except for the *su wx* starch, which had a much lower DP of 33). The high molecular weight peak maxima are interpreted to result from a dominant population of B2 chains. Most of the high molecular weight peak maxima are similar to those reported by Takeda (1988) for three dent maize samples (DP 45, 47, and 48).

For 14 of the samples, the mean retention time for the low molecular weight peak was 23.5 min, which corresponds to a DP of 10. The range was DP 10–12. Two values were not included in these calculations because the retention times of the main low molecular weight peaks were well below those for the other 14 starches. These values were for the *ae wx* starches from the S3-61 and W64A lines, which had retention times of 22.7 and 22.5 min, respectively, corresponding to DP 15 and 16. These latter values correspond well to those for B1 chains, as reported by Hizukuri (1986) for various nonmaize starches (DP 15–19). Chromatograms of the *ae wx* starches in the Ia453 and Ia5125 lines contained a shoulder strong enough to be recognized by the integrator at approximately the same retention time as the main low molecular weight peak for the other *ae wx* starches. Furthermore, chromatograms for all *ae du wx* starches contained this prominent, recognized shoulder. Thus, putative B1 chains were strongly evident for all *ae wx* and *du wx* starches. It is noteworthy that for these two *wx*-containing genotypes, but not other *wx*-containing genotypes, Boyer and Liu (1985) observed that a considerable fraction of the amylopectin was of lower than usual molecular weight. In the present work, the observed DP of 10–12 correspond to those observed by Hizukuri (1986) for the A chains for the nonmaize starches studied but are apparently shorter than those observed by Takeda et al (1988) for normal maize. Although no attempt was made to discuss A and B1 chains in that work, the chromatograms published by Takeda et al (1988) indicate that the B1 chains might be DP > 20 and the A chains ~ DP 17–18.

The relative areas of the two major chromatographic peaks varied considerably. Within each line, the *ae wx* starches had the highest proportion as the high molecular weight peak, at 50–64%. Within each line, the *du wx* starches had the lowest proportion as the high molecular weight peak (but not significantly so for the S3-61 line) at 29–38% from the Oh43 inbred line. Fuwa et al (1987) also observed a low proportion of material in the high molecular weight peak for debranched *du wx* starch. Most of the chromatograms had shoulders within one or both of the major peaks. Within each line, the *wx* and *ae wx* starches had higher molecular weight shoulders in the high molecular weight peak. The primary low molecular weight peak was accompanied by a secondary peak at slightly higher molecular weight in *ae du wx* starches from all four lines (Table IV) as well as in the *ae wx* starches from the Ia5125 and Ia453 lines. For the *ae wx* starches from the S3-61 and W64A lines, the low molecular weight peak was broad, spanning the region of the primary peak and

the shoulder or secondary peak in the other starch samples.

It appears that variation in the proportion of chains between and within the two major peaks, rather than translocation of the entire chain length distribution, may be responsible for variation in amylopectin structure. Variation in these proportions may help explain differences in physical properties of the starches.

By factorial analysis, the effect of genotype and line on the pattern of debranched material is highly complex, with all main effects and interactions influencing the percentage of the total area in the main peaks and the low molecular weight retention time. Although the main effects of *ae* and of inbred line on the high molecular weight retention time were highly significant, the main effect of *du* was not significant, possibly due to a highly significant interaction term, *du* × line. By examination of Table IV, it would appear that this significant interaction term results primarily from the data in the S3-61 line.

Relationship Between Gelatinization Behavior and Amylopectin Fine Structure

Within the Ia5125 line. Only the *wx*-containing genotypes were subjected to isoamylase debranching as well as to thermal analysis; consequently, discussion must be limited to seven starches from the Ia5125 line. For these starches the highest enthalpy, and one of the two highest T_{max} values, was observed for the *ae su wx* genotype. This sample also had the greatest proportion of chains in the high molecular weight peak (56%). The other sample with a high T_{max} was *ae wx*, yet enthalpy for this sample was 0.8 cal/g lower than for the *ae su wx*. It too had a relatively large proportion of chains in the high molecular weight peak (50%). Both samples had a slightly shorter retention time for the low molecular weight peak than for the others, corresponding to a slightly larger DP.

For one of the two starches with the lowest proportion of area in the high molecular weight peak, *du su wx*, enthalpy was the lowest. For the other, *du wx*, enthalpy was considerably higher. Other *wx*-containing samples within the Ia5125 line showed no consistent relationship between their gelatinization behavior and chain distribution. Overall, for samples from the Ia5125 line, there may be a weak relationship between fine structure parameters and thermal analysis parameters.

Starches from the four wx*-containing genotypes from the four lines.* Among these 16 starch samples, a pattern is observed in two respects. Within each line, the *ae wx* starches had the greatest proportion of area in the high molecular weight peak as well as the highest T_{max}. Secondly, the two samples with much shorter retention times for the primary low molecular weight peak, the *ae wx* in S3-61 and W64A, were the samples with the two highest enthalpies. These observations are consistent with the notion that a higher proportion of B2 and B3 chains to B1 and A chains may be responsible for a higher T_{max} but not necessarily a higher enthalpy. Because the much shorter retention time for the low molecular weight peak may be interpreted as due to a predominance of B1 over A chains (a reversal of the situation for most starches analyzed), it is plausible that the proportion of B1 to A chains is a strong determinant of enthalpy, possibly in combination with the proportion of the chains in the two major

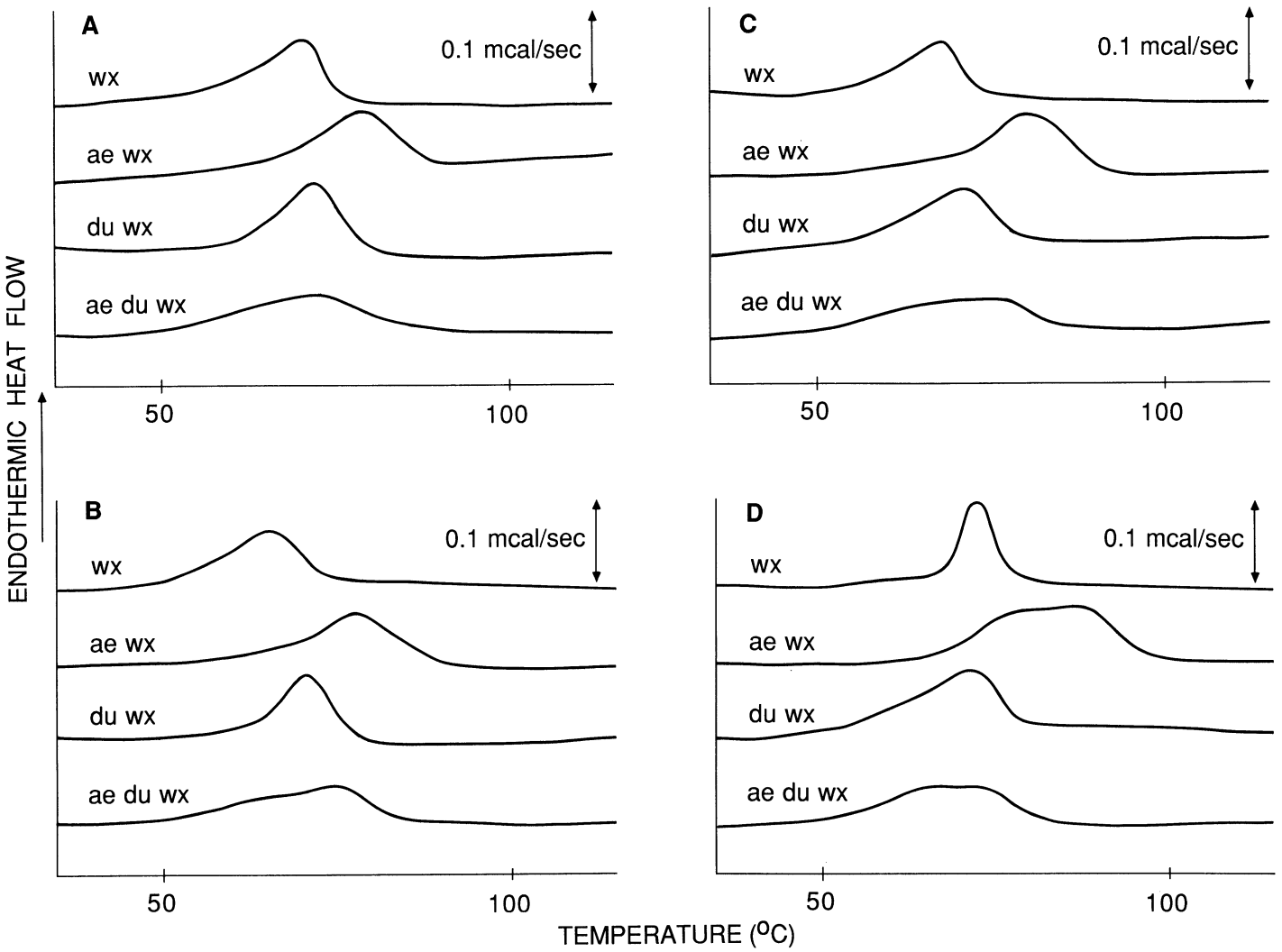

Fig. 3. Differential scanning calorimetry thermograms for four *wx*-containing maize genotypes as expressed in four inbred lines: Ia5125 (**A**), Ia453 (**B**), S3-61 (**C**), and W64A (**D**).

TABLE IV
Distribution of Linear Chains After Isoamylase Debranching and Size-Exclusion High-Performance Liquid Chromatography Analysis for *wx*-Containing Genotypes from Four Inbred Lines[a]

Starch[b]	High-MW[c] Peak		Retention Time (min)	
	Retention Time (min)	Percent of Total Area	Main Low-MW Peak	Secondary Low-MV Peak
Ia5125				
wx[d]	19.7 ± 0.1 fg	41 ± 1 e	23.5 ± 0.1 ab	none[e]
ae wx[d]	20.0 ± 0.1 bcde	50 ± 1 c	23.3 ± 0.1 c	22.4 ± 0.0
du wx[d]	19.8 ± 0.3 defg	38 ± 1 f	23.7 ± 0.2 a	none[e]
ae du wx[d]	20.3 ± 0.3 b	45 ± 0 d	23.7 ± 0.1 a	22.2 ± 0.1
Ia453				
wx	19.9 ± 0.2 cdef	42 ± 2 e	23.6 ± 0.0 ab	none[e]
ae wx	20.9 ± 0.1 a	64 ± 0 a	23.2 ± 0.0 c	22.6 ± 0.2
du wx	19.7 ± 0.1 fg	32 ± 1 i	23.6 ± 0.1 ab	none[e]
ae du wx	20.3 ± 0.1 b	36 ± 1 gh	23.6 ± 0.1 ab	22.3 ± 0.1
S3-61				
wx	19.7 ± 0.0 efg	41 ± 2 e	23.6 ± 0.0 ab	none[e]
ae wx	19.8 ± 0.2 defg	55 ± 0 b	22.7 ± 0.0 d	none[f]
du wx	20.1 ± 0.1 bcd	35 ± 1 h	23.7 ± 0.2 a	none[e]
ae du wx	20.2 ± 0.0 bc	36 ± 0 h	23.6 ± 0.1 ab	22.3 ± 0.0
W64A				
wx	19.8 ± 0.2 defg	41 ± 2 e	23.5 ± 0.1 b	none[e]
ae wx	20.0 ± 0.3 bcd	62 ± 1 a	22.5 ± 0.0 e	none[f]
du wx	19.6 ± 0.3 g	29 ± 1 j	23.6 ± 0.0 ab	none[e]
ae du wx	20.2 ± 0.0 bc	38 ± 1 fg	23.6 ± 0.1 ab	22.4 ± 0.1

[a]Values are mean ± SD. In the same column, values followed by a common letter are not significantly different at $P < 0.05$, by LSD analysis. Pooled standard errors: high-MW retention time, 0.011; high-MW percent of total area, 0.399; low-MW retention time, 0.003.
[b]Genotype. Three replicate analyses for each genotype.
[c]MW = Molecular weight.
[d]Data also contained in Table II.
[e]No peak was identified by the integrator, but a shoulder was present in this region.
[f]For this sample, the primary peak occurred at a retention time approximately that observed for the secondary peak for 14 of the 16 samples.

TABLE V
Correlation Between HPLC[a] Size-Exclusion Chromatogram Parameters for Debranched Starch and DSC[b] Thermal Behavior Parameters for *wx*-Containing Genotypes from Four Inbred Lines

Parameters	Correlation
High-MW[c] peak retention time and ...	
T_o	−0.05
T_{max}	0.28
ΔH	−0.001
High-MW peak percent of total area and ...	
T_o	0.59
T_{max}	0.71
ΔH	−0.60
Low-MW peak retention time and ...	
T_o	−0.64
T_{max}	−0.85
ΔH	−0.88

[a]High-performance liquid chromatography.
[b]Differential scanning calorimetry.
[c]MW = Molecular weight.

peaks. Variation among amylopectin molecules may also contribute to observed thermal behavior of these starches. For the *ae wx* genotype, the line (Ia5125) with the greatest proportion of low molecular weight amylopectin (Boyer and Liu 1985) had the lowest enthalpy.

Correlation analyses between the three HPLC chromatogram parameters and the three DSC thermogram parameters are shown in Table V. It is clear that the retention time of the high molecular weight peak did not correlate well with any DSC parameter. On the other hand, the percent of total area in the high molecular weight peak correlated positively with each DSC parameter, and a negative correlation of similar magnitude was observed between the retention time of the low molecular weight peak and each DSC parameter. Thus, it would appear that the thermal behavior of the starches might be related to two aspects of the amylopectin structure: more chains of higher molecular weight, and a slightly longer population of lower molecular weight chains. Both would contribute to the previously observed longer average chain lengths for *ae wx* starches. It should be noted that the strength of the above correlations results disproportionately from the different behavior of the *ae wx* starches relative to that for the other starches.

That T_{max} and enthalpy might be a function of both aspects of amylopectin structure may be explained based on the crystallite structure of the granule. According to the several cluster models of amylopectin, many crystallites are linked due to the inclusion of at least one chain in contiguous crystallites. If the proportion of chains common to contiguous crystallites were greater (i.e., more B2 chains), the stability of each crystallite would become increasingly influenced by the behavior of its partner. Due to the increased supracrystallite order, it would take more thermal energy to overcome the kinetic barrier to gelatinization. The higher enthalpy would result from the increase in the total noncovalent forces of attraction of the linear chains. In Hizukuri's model (1986) the B1 chains are thought to be longer than the A chains, and both are thought to be localized to one crystallite region. The length of a crystallite would be longer (in the axis of the 1-4 linkages) if it were made up solely of B1 chains than if it were solely A chains. A higher proportion of B1 chains would result in slightly longer crystallites and at the same time increase the efficiency of the packing within the crystallite building blocks, leading to a proportionately greater fraction of the starch in crystalline form. On this basis as well, gelatinization T and enthalpy would be higher.

These conclusions appear reasonable for all four genotypes examined in the four lines; however, within the Ia5125 line for a greater variety of genotypes, including several containing the *su* gene, the relationship may be more complex. Continued research is needed to understand the way genotype and line might influence amylopectin structure and consequently alter starch physical behavior. One aspect of amylopectin structure not addressed in the present work is molecular weight, which has been shown to be unusually disperse for starches from the *ae wx* and *ae du wx* genotypes (Boyer and Liu 1985). On another level, the influence of genetic background on granule behavior may be partially mediated through variation in granule

architecture, size, or morphology. Considerable research remains to be done to address the complex relationship between genetic background and starch physical behavior.

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LITERATURE CITED

BOYER, C. D., GARWOOD, D. L., and SHANNON, J. C. 1976. The interaction of the amylose-extender and waxy mutants of maize (*Zea mays* L). Fine structure of amylose-extender waxy starch. Staerke 28:405.

BOYER, C. D. and LIU, K. C. 1985. The interaction of endosperm genotype and genetic background. Part I. Differences in chromatographic profiles of starches from nonmutant and mutant endosperms. Starch/Staerke 37:73.

BROCKETT, E., THOMPSON, D., DAVIS, T., and BOYER, C. D. 1988. Gelatinization characteristics of starch from *du, wx, ae*, and *ae wx* endosperm of sweet corn inbred Ia5125. Starch/Staerke 40:175.

DONOVAN, J. W. 1979. Phase transitions of the starch-water system. Biopolymers 18:263.

FRENCH, D. 1972. Fine structure of starch and its relationship to the organization of starch granules. J. Jpn. Soc. Starch Sci. 19:8.

FUWA, H., GLOVER, D. V., MIYAURA, K., INOUCHI, N., and SUGIMOTO, Y. 1987. Chain length distribution of amylopectins of double- and triple-mutants containing the waxy gene in the inbred Oh43 maize background. Starch/Staerke 9:295.

HAWORTH, W. N., HIRST, E. L., and ISHERWOOD, F. A. 1937. Polysaccharides, Part XXII. Determination of the chain length of glycogen. J. Chem. Soc. (London) p. 577.

HIZUKURI, S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. Carbohydr. Res. 147:342.

HODGE, J. C., and HOFREITER, B. T. 1962. Determination of reducing sugars and carbohydrates. Page 380 in: Methods in Carbohydrate Chemistry, vol 1. R. L. Whistler and M. L. Wolfrom, eds. Academic Press: New York.

INOUCHI, N., GLOVER, D. V., and FUWA, H. 1987. Chain length distribution of amylopectins of several single mutants and the normal counterpart, and sugary-1 phytoglycogen in maize (*Zea mays* L.). Starch/Staerke 39:259.

KOBAYASHI, S., SCHWARTZ, S. J., and LINEBACK, D. R. 1986. Comparison of the structures of amylopectins from different wheat varieties. Cereal Chem. 63:71.

KRUEGER, B. R., KNUTSON, C. A., INGLETT, G. E., and WALKER, C. E. 1987. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. J. Food Sci. 52:715.

LII, C. Y., and LINEBACK, D. R. 1977. Characterization and comparison of cereal starches. Cereal Chem. 54:138.

LUND, D. 1984. Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. CRC Crit. Rev. Food Sci. Nutr. 20:249.

MEYER, K. H., and BERNFELD, P. 1940. Recherches sur l'amidon V. L'amylopectine. Helv. Chim. Acta 23:875.

NIKUNI, Z. 1969. Science of Cookery (in Japanese) 2:6. (Original not seen, cited in NIKUNI 1978).

NIKUNI, Z. 1978. Studies on starch granules. Starch/Staerke 30:105.

PEAT, S., WHELAN, W. J., and THOMAS, G. J. 1952. Evidence of multiple branching in waxy maize starch. J. Chem. Soc. (London) p. 4546.

PORRO, M., VITI, S., ANTON, G., and NERI, P. 1981. Modifications of the Park-Johnson ferricyanide submicromethod for the assay of reducing groups in carbohydrates. Anal. Biochem. 118:301.

ROBIN, J. P., MERCIER, C., and GUILBOT, A. 1974. Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. Cereal Chem. 51:389.

SHANNON, J. C., and GARWOOD, D. L. 1984. Genetics and physiology of starch development. In: Starch: Chemistry and Technology. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

STAUDINGER, H., and HUSEMAN, E. 1937. Uber hochpolymere verbindungen. Uber die konstitution der starke. Justus Liebigs Annal. Chem. 527:195.

TAKEDA, Y., SHITAOZONO, T., and HIZUKURI, S. 1988. Molecular structure of corn starch. Starch/Staerke 40:51.

WHISTLER, R. L., and DANIEL, J. R. 1984. Molecular structure of starch. In: Starch: Chemistry and Technology. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

WOOTEN, M., and BAMUNUARACHCHI, A. 1979. Application of differential scanning calorimetry to starch gelatinization. I. Commercial native and modified starches. Starch/Staerke 31:201.

YOKOBAYASHI, K., MISAKI, A., and HARADA, T. 1970. Purification and properties of Pseudomonas isoamylase. Biochim. Biophys. Acta 212:458.

ZOBEL, H. F. 1984. Gelatinization of starch and mechanical properties of starch pastes. In: Starch: Chemistry and Technology. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

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