Fine Structure of Amylopectin in Relation to Gelatinization and Retrogradation Behavior of Maize Starches from Three *wx*-Containing Genotypes in Two Inbred Lines

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ABSTRACT

Maize starches from three *wx*-containing genotypes (*wx, du wx,* and *ae wx*) in two inbred lines (W64A and Ia5125) were examined to evaluate the effect of mutant genotype on amylopectin fine structure and thermal behavior of starch granules. The amylopectin chain length distribution and A:B chain ratio were investigated by enzymatic treatments followed by high-performance size-exclusion chromatographic separation. Gelatinization and retrogradation behavior at 10 and 30% starch concentrations were studied by differential scanning calorimetry. Chain length distribution profiles of the isoamylase-debranched amylopectins showed distinct patterns according to genotypes. Starch from the *ae wx* genotype in both lines had a greater proportion of longer chains (degree of polymerization > 30) than the other genotypes. The A:B chain ratios were calculated from the high-performance size-exclusion chromatograms of the debranched β-limit dextrins. Except starch from the *ae wx* genotype of the W64A line, which had an A:B chain ratio of 1.5, all of the samples had A:B chain ratios within the range of 0.9–1.1. The A:B chain ratio of 1.5, together with the high proportion of longer chains in that sample, is consistent with the interpretation that the population of A chains in this sample contains some unusually long chains. In both lines, the starch granules from the *ae wx* genotype had the highest ΔH and T_{max} for both gelatinization and retrogradation. The high ΔH for the *ae wx* starches might be attributed to greater amounts of longer chains in these amylopectins. Except the starches from the *wx* genotype at 10% concentration, all of the gelatinized starch samples showed a differential scanning calorimetry endothermic peak after being stored at 4°C for seven days. Retrogradation ΔH was highest for the *ae wx* starches, both in absolute terms and as the proportion of gelatinization ΔH. In most cases retrogradation ΔH was higher (on dry weight basis) for 30% than for 10% starch; for the *du wx* starches, the ΔH at 30% starch was approximately twice that at 10%.

Corn starch is widely used in various food systems as a filling, thickening, or stabilizing agent. Specialty starches have been produced to meet the needs of new product formulations. New modification processes, and possibly new raw materials, are required to produce novel specialty starches such as starch-based fat replacers. New modification processes are limited by strict food regulations; therefore, the use of new raw material is more feasible for developing new starch products. To date waxy maize starch is probably the most important raw material for modified starches. Starches from other corn mutants with unique properties can also serve as raw materials for modification. For example, the modification of dull waxy starch (De Boer 1991) leads to properties not attainable by modification of waxy starch. Characterization of the physical and structural properties of the starches from different mutants is essential for the use of these starches as possible raw materials.

The effects of different mutant genotypes on the proportion of amylose and amylopectin of starch is well documented (Shannon and Garwood 1984). The structure of amylopectins is also affected by the endosperm genotype (Baba and Arai 1984, Boyer and Liu 1985, Inouchi et al 1987, Sanders et al 1990). Since amylopectin is the major component of most starches, variation in amylopectin structure can result in starch granules with very different physical properties. The structure of amylopectin has been studied by examining the chain profile and determining the A:B chain ratio, among other methods. Examination of the chain profile involves the complete debranching of amylopectin by pullulanase (Lee et al 1968) or isoamylase (Akai et al 1971) followed by the separation of linear chains by size-exclusion chromatography. The resultant chromatogram reveals the size distribution of the chains in the native amylopectin. This chain profile technique has been refined by Hizukuri (1986), who used a high-performance size-exclusion chromatography (HPSEC) system to increase the resolution. Sanders et al (1990) used HPSEC to show the diversity of chain length distributions of amylopectins from various corn mutants. The A:B chain ratio of amylopectin can be determined from the molar ratio of maltose and maltotriose to other linear fractions of debranched β-limit dextrin (Peat et al 1956). The maltose and maltotriose represent the A chains of the native amylopectin. Alternatively, Hizukuri (1986) estimated the A:B chain ratios for several starches from the polymodal chain length distributions of debranched amylopectins.

Gelatinization and retrogradation are two important physical behaviors of starch. Starch gelatinization is the process that takes place when starch granules are heated in the presence of water, resulting in the disruption of molecular order within the starch granule. The process is manifested by irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization (Atwell et al 1988). Gelatinization is required to produce the desired functionality (i.e., thickening and swelling) of starch in a food system. Starch retrogradation is a process that occurs when the molecules composing gelatinized starch begin to reassociate, leading to a more ordered structure. Under favorable conditions, the ordered structure may develop into crystalline forms (Atwell et al 1988). Retrogradation is related to the stability of a starch paste during storage. The retrogradation of amylopectin is partially responsible for the staling of bread and other baked foods (McIver et al 1968, Cowell et al 1969). A widely used method for studying gelatinization and retrogradation is differential scanning calorimetry (DSC) (Atwell et al 1988). The advantage of DSC is that it provides not only the temperature but also the enthalpy change for a thermal transition. Another advantage of DSC is the small sample requirement for analysis.

The objective of this study was to investigate the variation in amylopectin structure, as affected by endosperm mutants, and the thermal behavior of the starch granules. To avoid the influences of amylose, starches from *wx*-containing genotypes were used in the study since they contain virtually 100% amylopectin (Shannon and Garwood 1984).

MATERIALS AND METHODS

Starch Samples

Backcross conversions for the endosperm mutants *waxy* (*wx*), *amylose-extender* (*ae*), 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

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were verified by appropriate allele tests (Boyer and Hannah, in press). Genotypes were identified by those gene loci homozygous for recessive mutant alleles. Other gene loci not listed were therefore homozygous for the dominant allele.

Starch samples from three genotypes (*wx, du wx,* and *ae wx*) in two inbred lines (W64A, a dent corn, and Ia5125, a sweet corn) examined in this research were isolated in the laboratory as described by Boyer and Liu (1985). These starches were assumed to contain 10% moisture.

Thermal Analysis of Gelatinization and Retrogradation

Gelatinization and retrogradation behaviors of two starch-water mixtures (10 and 30% starch content, dry weight basis) for each sample (at least three replicates for each sample) were studied using a differential scanning calorimeter (DSC-4, Perkin-Elmer, Norwalk, CT). Temperature and enthalpy of the instrument were calibrated using an indium standard.

Gelatinization

Approximately 2.0 mg of starch sample was placed in a pre-weighed coated aluminum pan (900796.901, Du Pont, Wilmington, DE) and the precise weight (±0.01 mg) was determined using an autobalance (AD2B, Perkin-Elmer). The calculated amount of deionized water was then added to adjust starch content to 10 and 30%, respectively. The starch slurry was hermetically sealed and allowed to equilibrate for 1 hr. The sample was then heated from 20 to 110°C at a heating rate of 10°C/min. The reference pan contained approximately 2.0 mg of freshly gelatinized waxy starch gel of either 10 or 30% starch content, according to the sample. The thermograms were analyzed on the thermal analysis data station of the DSC system. The enthalpy (ΔH, expressed on dry starch basis), onset temperature (T_o), and peak maximum temperature (T_{max}) were reported for each thermogram. The gelatinized starch sample (in the original sealed pan) was then stored at 4°C for the later retrogradation experiment.

Retrogradation

After storage at 4°C for seven days, the previously gelatinized starch sample was removed from the refrigerator and allowed to equilibrate at room temperature for 1 hr. Thermal analysis of the sample was then monitored by DSC using the same heating parameters as for the gelatinization process. The reference was prepared the same way as described in the previous section.

Statistics

Analysis of variance was performed on the data from thermal analysis. Multiple comparisons were performed by least significant difference after a preliminary F test.

Fine Structure of Amylopectin

Isoamylase debranching of amylopectin. Starch sample (10 mg) was dispersed in 2 ml of 90% (v/v) dimethyl sulfoxide (DMSO; molecular biology grade, Fisher Scientific, Fair Lawn, NJ) by heating in a boiling water bath for 10 min. After cooling, 6 ml of methanol was added, and the dispersion was mixed thoroughly and immersed in an ice bath for 30 min to facilitate precipitation of amylopectin. The sample was then centrifuged at 250 × g for 5 min, and the supernatant was decanted. To the amylopectin precipitate was added 2 ml of 0.05M sodium acetate buffer (pH 3.5), and the mixture was heated in a boiling water bath for 5 min. After cooling, 0.2 ml of isoamylase (EC 3.2.1.68; product number I 2758, Sigma Chemical Co., St. Louis, MO) solution (83 units/ml of 0.05M sodium acetate buffer, pH 3.5) was added, and the sample was incubated at 37°C in a water bath shaker for 24 hr. Under these conditions, the amylopectin was debranched completely, as demonstrated in preliminary work by the total conversion of the debranched materials to maltose upon digestion with β-amylase.

Debranching of amylopectin β-limit dextrin. The digestion procedure was adapted from Asaoka et al (1985) with slight modification. A 2-ml amylopectin dispersion (in 0.02N sodium acetate buffer, pH 4.8) was prepared by the same procedure used for isoamylase digestion, except 20 mg instead of 10 mg of starch was used. The amylopectins were digested with 0.2 ml of β-amylase (EC 3.2.1.2; Sigma product number A 7005) solution (42 units/ml of 0.02N sodium acetate buffer, pH 4.8) at 37°C. After 48 hr, the digest was heated in a boiling water bath for 10 min, and another 0.2 ml of β-amylase solution was added after cooling. The digestion was complete after another 48 hr of incubation at 37°C. The digest was then heated in boiling water for 10 min to inactivate the enzyme. The β-limit dextrins were precipitated by the addition of 3 vol of methanol. After settling in an ice bath for 30 min, the β-limit dextrin precipitates were separated from the soluble maltose by centrifuging for 5 min at 250 × g. The β-limit dextrins were then washed three times with 2 ml of 75% methanol and air-dried.

The resulting β-limit dextrins were dispersed in 1 ml of 0.05N sodium acetate buffer, pH 5.2, by heating in a boiling water bath for 5 min. After cooling, the dispersion was incubated with 0.2 ml of the isoamylase solution at 37°C for 24 hr. The digest was later heated in a boiling water bath for 10 min to inactivate the enzyme and then, after cooling, incubated with 0.2 ml of pullulanase (EC 3.2.1.41; Sigma product number P 5420) solution (2 units/ml of 0.05N sodium acetate buffer, pH 5.2) at 37°C for 24 hr. The enzyme was inactivated by heating the digest in a boiling water bath for 10 min.

Separation by HPSEC. The HPSEC system consisted of a pump (M-45, 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, Du Pont) packed with small (5–7 μm), porous silica microspheres, and a differential refractometer (RI detector, model 401, Waters). Detector responses were transformed into digital signals by a D/A converter and then analyzed using Chromatochart software (Interactive Microware, State College, PA) on an Apple IIe computer.

The samples for injection were prepared as follows: a 0.1-ml aliquot of the digestion mixture was removed and 0.9 ml of DMSO added to make a 90% DMSO solution. This DMSO solution was heated in boiling water for 10 min in a capped 1.5-ml micro-centrifuge tube and then centrifuged for 5 min at 2,500 × g. A 200-μl sample of the supernatant was injected into the HPSEC system. The flow rate was 0.4 ml/min. The mobile phase (pure DMSO) had been filtered through a 0.45-μm nylon membrane filter and then degassed with a vacuum suction device. The columns and the detector were maintained at 35 and 30°C with a column heater (model 1102/CX-4, Eldex Laboratories, Menlo Park, CA) and a circulating water bath (model FE 2, HAAKE, Germany), respectively.

A calibration curve for the chromatograms was constructed using pullulan standards (Hayashibara, Japan; purchased through Waters) and partially acid-hydrolyzed starch. A 100-μl aliquot of the partially acid-hydrolyzed starch (1 mg/ml in 90% DMSO) was injected into the HPLC system under the same conditions as described above. The resulting chromatogram showed eight partially resolved peaks corresponding to degree of polymerization (DP) from 1 to 8. Two pullulan standards (20 μl; 1 mg/ml) with nominal molecular weights of 5,800 (P-5) and 12,200 (P-10) Da also were injected separately under the same conditions. The molecular weights and the corresponding retention times of the two pullulan standards and the seven standards (DP 2–8) from the partially hydrolyzed starch were used to construct the calibration curve with a cubic fit using the Chromatochart software.

The chromatograms shown in the results were derived from the originally recorded data as follows. From the Chromatochart software, a set of data consisting of elution time, RI detector response, and molecular weight (based on the calibration curve) was recorded for each chromatogram. The RI response is proportional to the mass of the eluted material. Another set of data, representing the relative mole content, was derived by dividing the relative mass (i.e., the RI detector response) by the corresponding molecular weight. The data were then transformed into the chromatogram by plotting both the RI detector responses and the relative mole contents versus the corresponding elution

times (Cricket Software, Malvern, PA). For each chromatogram, the peak DP, number-average DP ($\overline{DP}_n$), weight percentage, and mole percentage of each fraction were reported. The weight percentage and $\overline{DP}_n$ were calculated by the Chromatochart program. Peak DP of a fraction was identified from the Chromatochart data as the DP at which the corresponding RI response was greatest. For each fraction, the ratio of weight percentage to $\overline{DP}_n$ was calculated to obtain what we term the relative mole content (not reported) of the fraction. The mole percentage of a fraction was then obtained from the ratio of relative mole content of the fraction to the sum of the relative mole contents of all fractions.

Each sample was digested on two separate occasions, and derived data presented are the mean for the two digestions.

RESULTS

Fine Structure of Amylopectin

Debranching of amylopectin. Representative chromatograms for the isoamylase-debranched starches of the *wx*-containing genotypes from the Ia5125 and the W64A inbred lines are shown in Figure 1. The shapes of the chromatograms varied among the three genotypes. For each genotype, the debranched starches from the two inbred lines had a similar elution pattern. For the *wx* and *ae wx* genotypes in both lines, the chromatograms are bimodal. For the *du wx* genotype in each line, the chromatograms seem to be trimodal. The dividing lines for three fractions, high molecular weight (HMW), intermediate molecular weight (IMW), and low molecular weight (LMW), are drawn at minimum and inflection points of the chromatograms at DP 31 and 16. For comparison, these DP values were also used to divide the *wx* and *ae wx* genotypes into three fractions. The chain length distribution parameters for the chromatograms are presented in Table I.

Chains in the LMW fraction made up the major portion of the total number of chains of the debranched amylopectins for all six samples (Table I). In both lines, the mole percentage of the LMW fraction of the *wx* and *du wx* genotypes (about 70%) was higher than that (about 50%) for the LMW fraction of the *ae wx* genotype. The mole percentage of the HMW fractions (15–19%) for the *ae wx* genotypes were higher than those (5–7%) for the other genotypes.

Debranching of β-limit dextrins. Representative chromatograms of the debranched β-limit dextrins from the *wx*-containing genotypes in the Ia5125 and W64A lines are shown in Figure 2. All chromatograms were divided into three fractions (I–III). Fraction I represents the B chains in the original amylopectins, and fractions II and III represent the A chains.

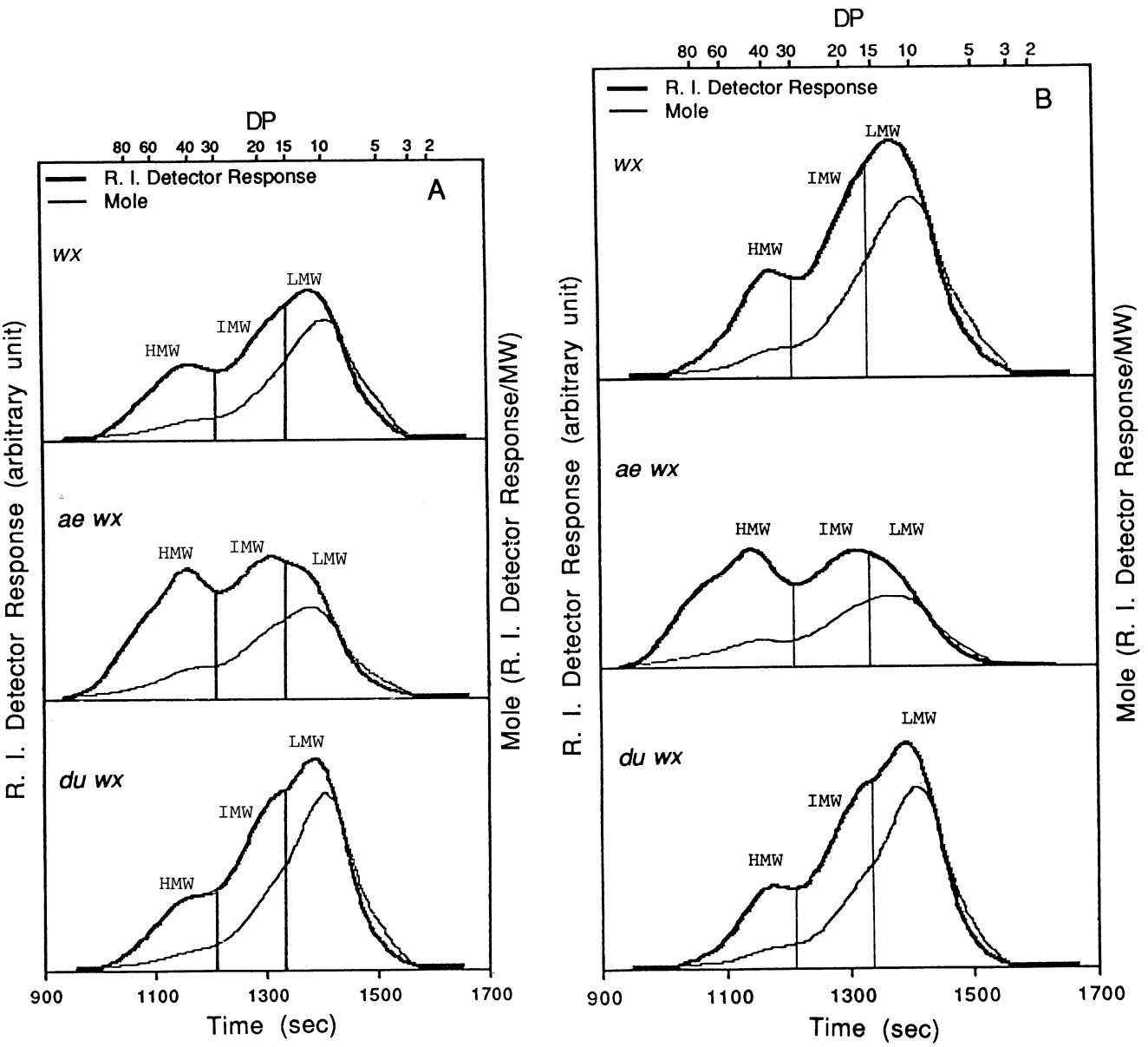

Fig. 1. Size-exclusion high-performance liquid chromatograms of the debranched starches from the *wx*-containing genotypes. **A,** Ia5125 inbred line; **B,** W64A inbred line. HMW, IMW, and LMW = high, intermediate, and low molecular weight, respectively; DP = degree of polymerization.

The demarcations for the three fractions are drawn at the minima between DP 2 and 3 and between DP 3 and 4. The demarcations for the *wx* genotype of the W64A line were used for those chromatograms that do not have a minimum between DP 2 and 3 or DP 3 and 4. The A:B chain ratio was calculated from the mole percentage figures as (II + III)/I. Except the *ae wx* genotype of the W64A inbred line, which had an A:B chain ratio of 1.5, all other samples had an A:B chain ratio within the range of 0.9–1.1. Table II summarizes the chain profile parameters for the debranched β-limit dextrins.

Thermal Analysis of Gelatinization and Retrogradation

Gelatinization. For each genotype, the shape and the parameters of the gelatinization thermogram were similar at the two starch concentrations (10 and 30%). Representative DSC thermograms for gelatinization of the starches from the Ia5125 line and the W64A line are shown in Figure 3 for the 30% starch concentration only. The T_o, T_{max}, and ΔH are reported in Table III. Except the *ae wx* genotype from the W64A line, which had a bimodal endothermic peak, all of the other thermograms had only one endothermic peak. For the *ae wx* genotypes in both lines, the endothermic peak was much broader than those for the other genotypes. The *wx* genotype from the W64A line had the sharpest endothermic peak among all of the starches. The endothermic peak for the *du wx* genotype from the W64A line had a low temperature shoulder that was not observed in the other genotypes or in the Ia5125 line.

The T_o ranged from 58.4 (*wx*) to 66.8°C (*ae wx*) for the Ia5125 line and from 60.3 (*du wx*) to 67.8°C (*ae wx*) for the W64A line. Within each line, the thermogram for the *ae wx* genotype had the highest ΔH (79.9 and 80.0°C for the Ia5125 and W64A lines, respectively). In each line the starch had the lowest T_{max} (66.9 and 68.7°C for the Ia5125 and W64A lines, respectively). Among the genotypes in each line, the *ae wx* starches had the highest ΔH (4.6 and 4.2 cal/g for the W64A and Ia5125 lines, respectively) and the *wx* starches had the lowest (3.1 and 3.4 cal/g for the Ia5125 and W64A lines, respectively).

Retrogradation. Representative DSC thermograms for the aged starch gels are shown in Figure 4, and the T_o, T_{max}, and ΔH for the thermograms are reported in Table IV. The shapes of the thermograms varied among the samples. The T_o and T_{max} were about 20°C lower and the peaks were broader for the retrogradation thermograms compared with those for gelatinization. The T_o for all of the thermograms was similar. The T_{max} for the *ae wx* genotypes was significantly higher than that for the other genotypes.

Starch concentration was an important factor influencing the shapes and enthalpies of the retrogradation thermograms, especially for starches from the *wx* and *du wx* genotypes. This concentration dependency was not observed in gelatinization. For the *wx* genotype in both lines, no retrogradation endothermic peak was observed for the 10% starch gel. All of the starch gels of the *ae wx* starches from the two lines had very broad endothermic peaks, which spanned a temperature range of about 50°C.

Excluding the 10% *wx* starch gels, which showed no sign of retrogradation in either line, the ΔH for the retrograded gels varied from 4.3 to 1.3 cal/g (dry weight basis). Within a line and at a given concentration, the ΔH increased in the order of *wx, du wx,* and *ae wx*. In both inbred lines, the retrogradation ΔH for the *du wx* genotype more than doubled when the gel concentration was increased from 10 to 30%. On the other hand, the concentration had no significant effect on ΔH for the *ae wx* genotypes: ΔH was virtually the same for the Ia5125 line (4.3 and 4.2 cal/g for the 30 and 10% gels, respectively) and differed slightly for the W64A line (4.2 and 3.6 cal/g for the 30 and 10% gels, respectively).

TABLE I
Characterization of Chain Length Distribution of Isoamylase-Debranched Starches from the *wx*-Containing Genotypes from the Ia5125 and W64A Lines[a]

Starch	Characteristic	Entire Sample	Fraction[b] HMW	IMW	LMW
Ia5125 line					
wx	Peak DP[c]		40 (40, 39)	NP[d]	11 (11, 11)
	$\overline{DP}_n$[e]	14 (14, 14)	46 (46, 46)	20 (20, 20)	9 (9, 9)
	Weight, %		23.1 (23.6, 22.6)	33.1 (34.6, 31.6)	43.8 (41.8, 45.8)
	Mole, %		7.2	23.6	69.2
ae wx	Peak DP		41 (40, 42)	18 (18,18)	NP
	$\overline{DP}_n$	19 (19, 19)	49 (48, 49)	20 (19, 20)	10 (10, 10)
	Weight, %		37.9 (38.7, 37.1)	36.6 (35.4, 37.8)	25.5 (25.9, 25.1)
	Mole, %		15.0	36.0	49.0
du wx	Peak DP		NP	NP	11 (11, 11)
	$\overline{DP}_n$	14 (14, 14)	45 (44, 45)	20 (20, 19)	10 (10, 10)
	Weight, %		16.4 (16.9, 15.9)	33.2 (34.4, 32.0)	50.4 (48.7, 52.1)
	Mole, %		5.2	24.0	70.9
W64A line					
wx	Peak DP		36 (35, 36)	NP	12 (12, 12)
	$\overline{DP}_n$	14 (14, 14)	42 (42, 41)	19 (18, 19)	9 (9, 9)
	Weight, %		18.6 (18.1, 19.1)	34.5 (33.8, 35.2)	46.9 (48.1, 45.7)
	Mole, %		6.0	24.8	69.2
ae wx	Peak DP		44 (44, 44)	18 (18,18)	NP
	$\overline{DP}_n$	20 (19, 20)	49 (49, 48)	20 (19, 20)	10 (10, 10)
	Weight, %		45.2 (45.3, 45.1)	30.7 (29.6, 31.8)	24.1 (25.1, 23.1)
	Mole, %		19.0	32.0	49.0
du wx	Peak DP		38 (37, 38)	NP	11 (11, 11)
	$\overline{DP}_n$	14 (14, 14)	44 (43, 44)	21 (20, 21)	10 (10, 10)
	Weight, %		14.4 (15.0, 13.8)	33.6 (35.1, 32.1)	52.0 (49.9, 54.1)
	Mole, %		4.6	22.9	72.5

[a]Values are means from chromatograms of two completed digestions. Value for each digestion is reported in parentheses.
[b]HMW, IMW, and LMW = high, intermediate, and low molecular weight, respectively.
[c]Degree of polymerization.
[d]No peak observed in this fraction.
[e]Number average degree of polymerization.

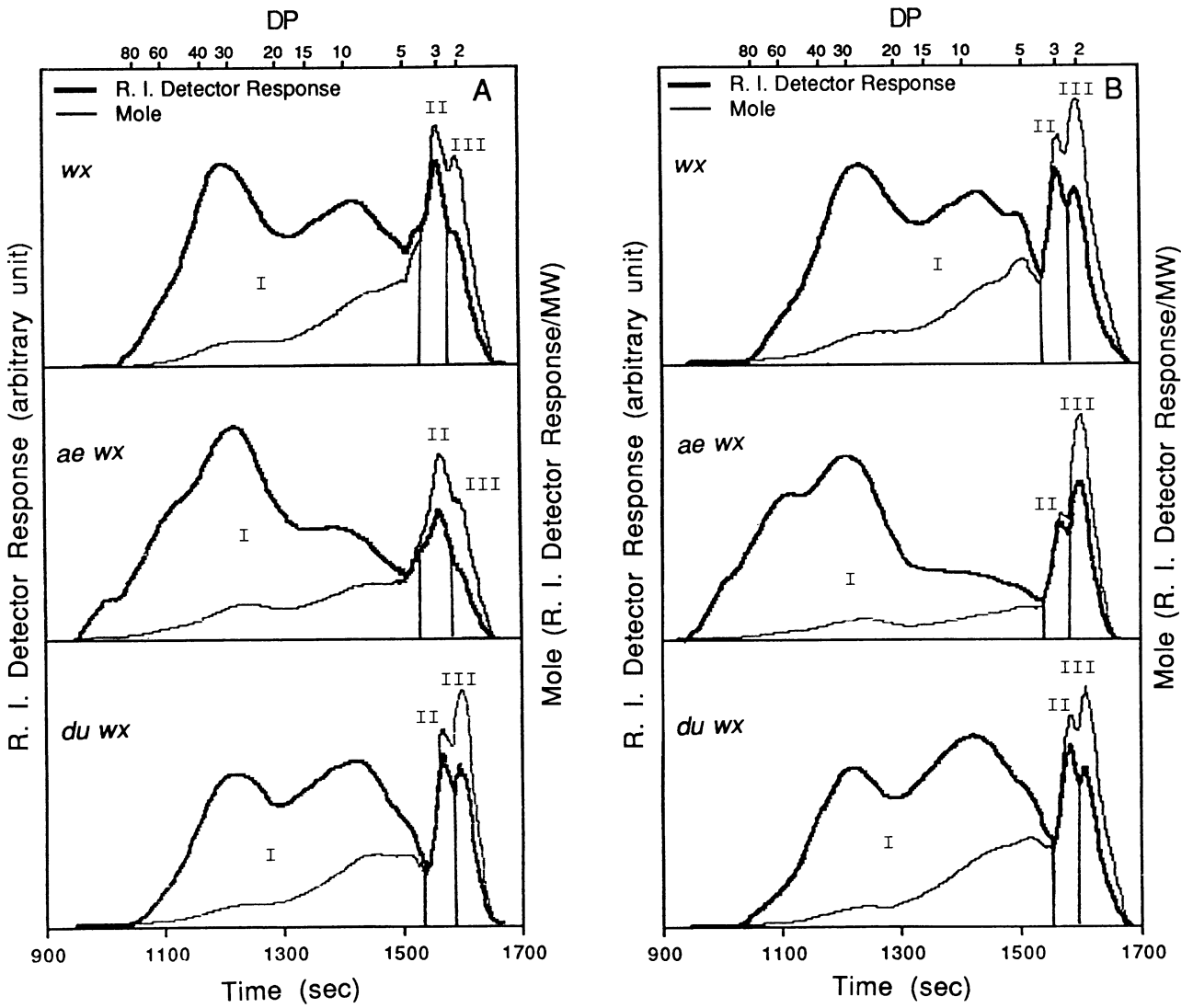

Fig. 2. Size-exclusion high-performance liquid chromatograms of the debranched β-limit dextrins from the *wx*-containing genotypes. **A,** Ia5125 inbred line; **B,** W64A inbred line. HMW, IMW, and LMW = high, intermediate, and low molecular weight, respectively; DP = degree of polymerization.

TABLE II
Characterization of Chain Length Distribution of Debranched β-Limit Dextrins from the *wx*-Containing Genotypes from the Ia5125 and W64A Inbred Lines[a]

Starch	Characteristic	Fraction I	Fraction II	Fraction III	A:B Chain Ratio
Ia5125 line					
wx	$\overline{DP_n}$[b]	14 (14, 13)	3 (3, 3)	2 (2, 2)	
	Weight, %	82.3 (83.0, 81.6)	12.1 (11.3, 12.9)	5.6 (5.7, 5.5)	
	Mole, %	46.4	31.7	21.9	1.1
ae wx	$\overline{DP_n}$	19 (19, 19)	3 (3, 3)	2 (2, 2)	
	Weight, %	86.9 (86.0, 87.8)	9.4 (10.5, 8.3)	3.7 (3.5, 3.8)	
	Mole, %	47.8	32.8	19.4	1.1
du wx	$\overline{DP_n}$	12 (12, 12)	3 (3, 3)	2 (2, 2)	
	Weight, %	82.5 (81.9, 83.1)	8.6 (8.8, 8.4)	8.9 (9.3, 8.5)	
	Mole, %	48.4	20.2	31.4	1.1
W64A line					
wx	$\overline{DP_n}$	13 (12, 13)	3 (3, 3)	2 (2, 2)	
	Weight, %	82.2 (81.5, 82.9)	8.4 (8.5, 8.3)	9.4 (10.0, 8.7)	
	Mole, %	45.8	20.2	34.0	1.1
ae wx	$\overline{DP_n}$	20 (20, 19)	3 (3, 3)	2 (2, 2)	
	Weight, %	84.7 (83.2, 86.2)	6.9 (7.5, 6.3)	8.4 (9.3, 7.5)	
	Mole, %	39.5	21.4	39.1	1.5
du wx	$\overline{DP_n}$	11 (11, 11)	3 (3, 3)	2 (2, 2)	
	Weight, %	83.3 (83.4, 83.2)	8.3 (8.5, 8.1)	8.4 (8.1, 8.7)	
	Mole, %	52.1	19.0	28.9	0.9

[a]Values are means from chromatograms of two completed digestions. Values for each digestion is reported in parentheses.
[b]Number average degree of polymerization.

DISCUSSION

Amylopectin Fine Structure Analysis

Chain profiles from the isoamylase-debranched amylopectins. For the Ia5125 and W64A lines, peak DP (when observed) for the HMW fraction ranged from 38 to 41 and from 36 to 44, respectively. These values are consistent with the peak DP values for B2 chains reported by Hizukuri (1986) for waxy rice (DP 41), tapioca (DP 38), kuzu (DP 39), and potato (DP 45). Among the genotypes in both lines, the *ae wx* genotype had the highest peak DP value for the HMW peak (see Table I). In both lines, the number of molecules in the HMW peak for the *ae wx* genotype was substantially higher than those for the other genotypes. The results indicate that the *ae* gene in the presence of *wx,* compared with the *wx* gene alone, had an effect of increasing the proportion of B2 and longer chains for the amylopectins. Sanders et al (1990) also reported an increase in HMW materials in the *ae wx* genotype.

The $\overline{DP}_n$ of the IMW fraction for all the samples was virtually the same at DP 20 (Table I). This value is consistent with the average chain lengths for the B1 chains reported by Hizukuri (1986) for kuzu (DP 20) and tapioca (DP 21), and slightly lower than that for waxy rice (DP 22) and potato (DP 24). Inouchi

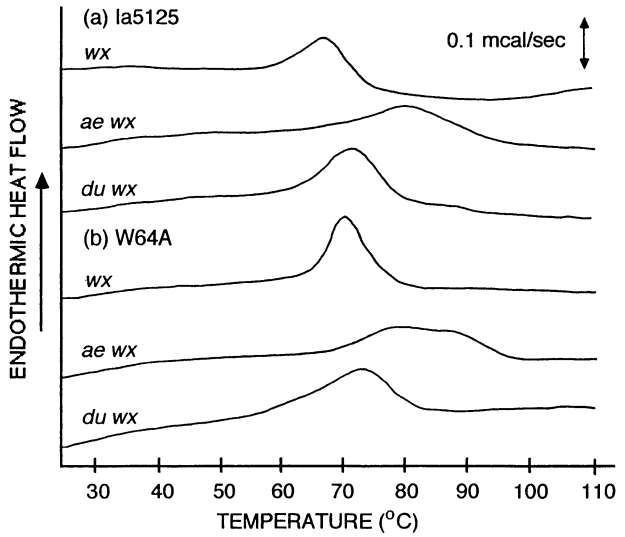

Fig. 3. Gelatinization differential scanning calorimetric thermograms for the starches from the *wx*-containing genotypes in the two inbred lines at 30% starch concentration.

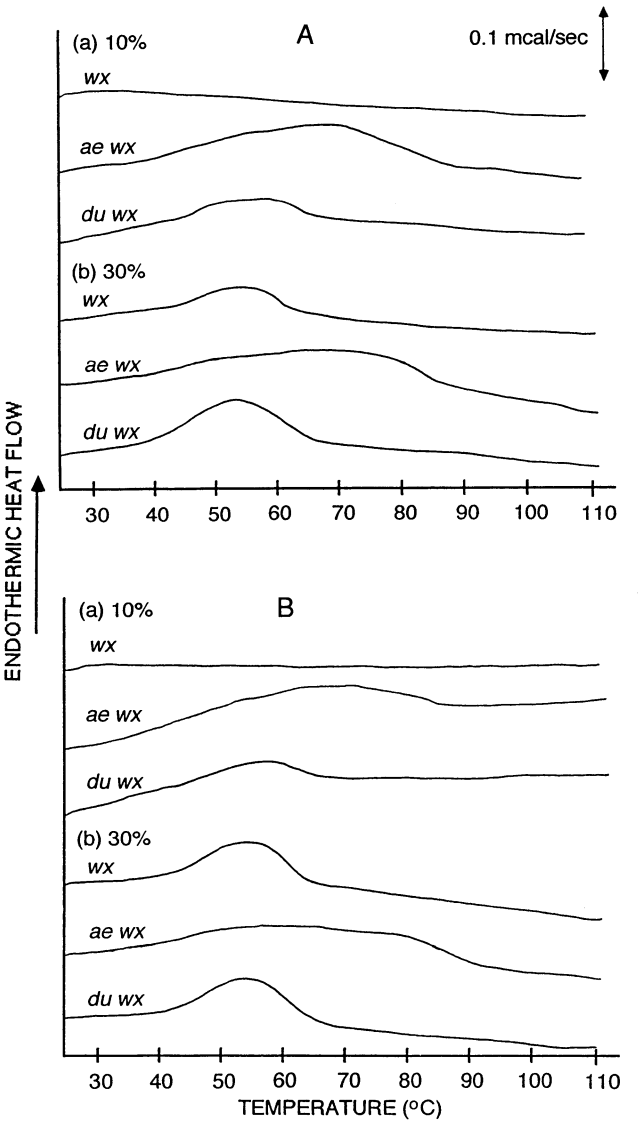

Fig. 4. Retrogradation differential scanning calorimetric thermograms for the starches from the *wx*-containing genotypes at 10 and 30% concentration. **A,** Ia5125 inbred line; **B,** W64A inbred line.

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

Starch[b]	Starch Content (%)	*n*	T_o (°C)	T_{max} (°C)	ΔH (cal/g)
Ia5125 line					
wx	10	5	58.6 ± 0.5 a	67.3 ± 0.3 a	3.0 ± 0.2 a
	30	5	58.4 ± 0.4 a	66.9 ± 0.5 a	3.2 ± 0.1 b
ae wx	10	3	66.8 ± 0.6 b	79.9 ± 0.6 b	4.2 ± 0.3 c
	30	4	65.6 ± 0.8 c	79.0 ± 0.6 b	4.1 ± 0.2 c
du wx	10	5	62.5 ± 0.9 d	71.0 ± 0.3 c	3.7 ± 0.0 d
	30	4	62.2 ± 0.4 d	70.0 ± 0.4 c	3.2 ± 0.2 b
W64A line					
wx	10	4	63.5 ± 0.1 e	68.7 ± 0.1 d	3.4 ± 0.1 e
	30	4	63.9 ± 0.4 e	69.0 ± 0.7 d	3.4 ± 0.1 e
ae wx	10	5	66.5 ± 0.2 b	80.0 ± 0.7 b	4.6 ± 0.2 f
	30	4	67.8 ± 0.2 f	79.3 ± 1.2 b	4.5 ± 0.2 f
du wx	10	5	60.8 ± 0.9 g	71.8 ± 0.2 e	3.6 ± 0.3 de
	30	5	60.3 ± 0.4 g	72.4 ± 0.4 e	3.6 ± 0.0 de

[a]Values are mean ± standard deviation. In the same column, values followed by a common letter are not significantly different at $P < 0.05$, by least significant difference analysis.
[b]Inbred line and genotype in two starch concentrations.

TABLE IV
Thermal Analysis of Retrogradation for Starches from the *wx*-Containing Genotypes from Two Inbred Lines[a]

Starch[b]	Starch Content (%)	*n*	T_o (°C)	T_{max} (°C)	ΔH (cal/g)
Ia5125 line					
wx	10	5	ND[c]	ND	ND
	30	5	41.4 ± 1.3 ac	54.0 ± 1.1 a	1.5 ± 0.2 a
ae wx	10	4	40.7 ± 0.8 ac	67.4 ± 0.6 b	4.2 ± 0.1 b
	30	4	40.6 ± 1.5 ac	65.4 ± 1.0 c	4.3 ± 0.2 b
du wx	10	4	42.3 ± 0.5 b	54.9 ± 0.7 a	1.3 ± 0.0 c
	30	5	40.3 ± 1.1 a	51.6 ± 0.9 d	2.9 ± 0.1 d
W64A line					
wx	10	4	ND	ND	ND
	30	5	40.6 ± 1.1 ac	52.6 ± 1.2 d	2.5 ± 0.1 e
ae wx	10	4	41.2 ± 1.5 ac	64.1 ± 2.6 ce	3.6 ± 0.1 f
	30	4	40.5 ± 0.8 ac	62.0 ± 0.5 e	4.3 ± 0.1 b
du wx	10	3	41.8 ± 0.8 c	56.6 ± 0.5 f	1.3 ± 0.1 c
	30	4	41.3 ± 0.9 ac	52.4 ± 0.6 d	3.0 ± 0.2 d

[a]Values are mean ± standard deviation. In the same column, values followed by a common letter are not significantly different at $P < 0.05$, by least significant difference analysis.
[b]Inbred line and genotype in two starch concentrations.
[c]Not detected.

et al (1987) reported DP 20.6 for the shorter B chains for waxy maize amylopectin. The mole percentage for the IMW fraction, as in the HMW fraction, was also higher for the *ae wx* genotype than for the other genotypes in both lines.

The peak DP of the LMW fraction for the *wx* and *du wx* genotypes studied ranged from 11 to 12 for both lines. In contrast, no peak was observed for the *ae wx* genotype in the LMW region. Instead, the peak occurred in the IMW region (at DP 18), and no more than a shoulder was observed in the LMW region. Hizukuri (1986) reported the peak DP values for A chains in the range of 11 (tapioca) to 16 (potato). The $\overline{DP}_n$ for the LMW fraction for the starches studied was either DP 9 (*wx*) or 10 (*ae wx* and *du wx*) in both lines. These values are smaller than the previously reported values for A chains from various sources. Inouchi et al (1987) proposed a DP of 12.4 for A chains for waxy maize amylopectins. The average chain lengths for A chains reported by Hizukuri (1986) ranged from DP 12 (tapioca) to 16 (potato). It appears that the discrepancy in the average chain length was due to the larger amounts of shorter chains ($DP < 10$) in the starches used in the present study. In Figure 1, the chromatograms indicate the presence of chains shorter than DP 5. These apparent short chains might come from the longer chains that eluted later than they should have as a result of band broadening in the columns. Since the calculation for $\overline{DP}_n$ is based on the chromatogram, these apparent short chains would result in a shorter $\overline{DP}_n$. This interpretation would account for the overall $\overline{DP}_n$ (14) being lower than previously reported values (24 and 19 by Peat et al 1956 and Inouchi et al 1987, respectively) calculated from chemical assay (i.e., total glucose units/total reducing groups of debranched amylopectin) for waxy maize. Hizukuri (1985) reported a weight-average chain length of DP 24 for waxy maize. According to that report, the ratio of the weight-average molecular weight to the number-average molecular weight (M_w/M_n) was 1.3; therefore, the $\overline{DP}_n$ for the waxy maize was close to 18 (24/1.3).

For the *ae wx* genotype in both lines, approximately 50% of the chains was found in the LMW fraction. On the other hand, approximately 70% of the chains was in this fraction for the *wx* and *du wx* genotypes (Table I). On the basis of the chromatograms of isoamylase-debranched amylopectins, Hizukuri (1986) reported that the A chains and the B1 chains constitute about 80% of the total chains for potato starch and about 90% for waxy rice, tapioca, and kuzu starches. In the present study, the combined percentages of LMW and IMW fractions (A and B1 chains) were nearly 80% for the *wx* genotypes and over 90% for the other genotypes. In terms of Hizukuri's cluster model, the *ae wx* genotype in both lines appears to have a greater proportion of B2 and longer chains than the other genotypes.

Brown et al (1971) reported that maize starches from several *ae*-containing genotypes, including *ae wx*, showed a B-type X-ray diffraction pattern. Hizukuri (1985) found that the X-ray diffraction pattern of a starch is related to the chain length distribution in the amylopectin. He reported that the amylopectin molecules of B-type starches have longer $\overline{DP}_n$ in both the long-chain fraction (which he termed F1) and the short-chain fraction (F2) than those of the A-type starches. He also found that the B-type starches have a smaller proportion of the short-chain fraction than the A-type starches. In the present study, the two *ae wx* starches produced a B-type X-ray diffraction pattern, whereas the *wx* and *du wx* starches showed an A pattern (Ian Harrison, The Pennsylvania State University, *unpublished data*). Consistent with the work of Hizukuri (1985), the combined proportion of the IMW and LMW fractions (analogous to Hizukuri's F2 fraction) of the *ae wx* starch is smaller than those of the A-type *wx* and *du wx* starches. The $\overline{DP}_n$ of the HMW (analogous to that of Hizukuri's F1) fraction is greater in the *ae wx* starch than the *wx* and *du wx* starches. The $\overline{DP}_n$ of the IMW and LMW fractions as a whole, which can be calculated from the weight percentage and $\overline{DP}_n$ of each individual fraction, is also greater in the *ae wx* starch than in the *wx* and *du wx* starches. In both lines, the $\overline{DP}_n$ of the combined fractions (IMW and LMW) is 14 for the *ae wx* and 12 for the *wx* and *du wx* genotypes. These results agree with the findings of Hizukuri (1985) and suggest that chain length distribution parameters of amylopectins contribute to the granular crystalline pattern of a starch. These differences in crystallite organization would be expected to affect the physical properties of the starch granules.

A:B chain ratio. The results are consistent with previously reported values for various starches. For waxy maize starch, the reported A:B chain ratios ranged from 1.0 (Manners and Matheson 1981) to 1.5 (Peat et al 1956), compared with 1.1 in the present study. The A:B chain ratio for waxy maize has been reported as high as 2.6 by Marshall and Whelan (1974). This value is higher than most reported values, and others have suggested that it might have resulted from experimental errors in the measurement of reducing power (Atwell et al 1980).

Hizukuri (1986) estimated the A:B chain ratios for waxy rice, tapioca, kuzu, and potato starches from the chromatograms of the isoamylase-debranched native amylopectins. He assigned each fraction of the chromatogram a letter designation and calculated the A:B chain ratio from the mole percentage of each fraction. The A:B chain ratios were 0.8–0.9 for kuzu and potato amylopectins, 1.5 for tapioca amylopectin (in that article it is reported as 0.89, which appears to be mistakenly calculated from the data for the kuzu amylopectin; from the original data for tapioca, the A:B chain ratio should be about 1.5), and 2.2 for waxy rice amylopectin. Applying the same approach to the chromatograms in the present work, the A:B chain ratios for the samples might be obtained from the mole percentage data listed in Table I, assuming the LMW fraction as the A chain fraction. Table V compares the A:B chain ratios calculated from the parameters for the debranched amylopectins (as by Hizukuri 1986) and from those for the debranched β-limit dextrins. Except for the *ae wx* genotype from the Ia5125 line, the A:B chain ratios calculated on the two bases are not in agreement. For the *wx* and *du wx* genotypes in both lines, the A:B chain ratio from the debranched native amylopectin is over twice that calculated from the debranched β-limit dextrin. The discrepancy might be because of the presence of shorter B1 chains included in the LMW fraction by the approach of Hizukuri (1986). These shorter B1 chains would erroneously have been considered A chains. The higher A:B chain ratio reported by Hizukuri (1986) (2.2 compared with 1.5 by Asaoka et al [1985]) for waxy rice amylopectin might also have resulted from the presence of short B1 chains in the A chain fraction.

For the *ae wx* genotype from the W64A line, the A:B chain ratio (1.0) calculated from the debranched native amylopectin is lower than that (1.5) calculated from the debranched β-limit dextrin. This difference in the A:B chain ratio could be explained by the presence of a population of unusually long A chains in the IMW fraction of the debranched native amylopectins. If they were erroneously considered as B chains, the A:B chain ratio would be underestimated. It is not clear why the aforementioned long A chain occurs in the W64A line but not in the Ia5125 line.

Using the mole percentage of each fraction in Table I, together with the A:B chain ratios from the debranched β-limit dextrins, it is possible to calculate the A:B1:(B2 and longer) chain ratios. The calculation, as shown below, is based on two assumptions:

TABLE V
Comparison of the A:B Chain Ratios Obtained from the Debranched Native Amylopectins and the Debranched β-Limit Dextrins

	A:B Chain Ratio	
Starch	Native Amylopectin	β-Limit Dextrin
Ia5125 line		
wx	2.3	1.1
ae wx	1.0	1.1
du wx	2.5	1.1
W64A line		
wx	2.3	1.1
ae wx	1.0	1.5
du wx	2.6	0.9

first, all A and B1 chains are present in the IMW and LMW fractions, and second, B2 and longer chains exist only in the HMW fraction. Data for the *wx* genotype of the Ia5125 line will be used as an example for the calculation of the A:B1:(B2 and longer) chain ratio, as follows. The first step in the calculation is to determine the A-chain percentage, which can be calculated from the A:B ratio of 1.1 for this genotype. Since $A/B = A/(1 - A) = 1.1$, A is solved to be 52%. From Table II, the combined mole percentage for the LMW and IMW fractions for the *wx* genotype is 93%. Since A = 52%, B1 = (93 − 52)% = 41%. The mole percentage for the B2 and longer chains is 7% (from the HMW fraction) according to the second assumption. The A:B1:(B2 and longer) chain ratio is therefore approximately equal to 52:41:7.

Using the same approach, the A:B1:(B2 and longer) chain ratios were calculated and listed in Table VI. For the *ae wx* genotype in both lines, the ratio of the B2 and longer chains to the B1 chains is much higher than the other genotypes. This result is consistent with the observation by Fuwa et al (1987), who reported that the amylopectin of the *ae wx* genotype in the Oh 43 inbred line had a higher proportion of long B chains as compared with that of *wx* amylopectin. They also found that the *du wx* amylopectin had a higher proportion of short B chains and a smaller proportion of long B chains than those of the *wx* genotype. The results in Table VI suggest that the *du wx* genotypes in the present study also had a higher proportion of B1 chains and a smaller proportion of B2 and longer chains.

Thermal Analysis of Gelatinization and Retrogradation

Gelatinization. For the *wx* genotype, the average gelatinization enthalpies (3.1 and 3.4 cal/g for the Ia5125 and W64A lines, respectively; Table III) are consistent with those reported by Sanders et al (1990) for the same inbred lines (3.4 and 3.0 cal/g at 10% starch concentration) and by Russell (1987) for commercial waxy starch (3.4 cal/g at 43% starch concentration).

Among the three genotypes in the same line, the *ae wx* genotype had the highest gelatinization temperature as well as the highest ΔH. The high gelatinization temperature might be due to the higher proportion of longer chains in the amylopectins from the *ae wx* genotypes. These chains could form long double helices (not necessarily entirely in crystallites) that would require a higher temperature to dissociate completely than that required for shorter double helices. The type of crystalline packing might also affect the gelatinization temperature of starch granules. Pfannemuller (1987) showed that maltooligomers of DP 10–12 formed A-type crystalline precipitates from pure aqueous solution at ambient or lower temperature, whereas those of DP 13–35 formed B-type crystals. This finding suggests that the length of the crystallites in a B-type starch would be longer than those in an A-type starch. The difference in crystallite length might partly account for the gelatinization temperature of the B-type *ae wx* starches being higher than those of the A-type *wx* and *du wx* starches.

The broad endotherms of the *ae wx* starches might indicate a lack of homogeneity of ordered structures inside the granules. These granules might contain crystallites and double helices (which are not in crystalline form) of widely varying length, resulting in a broad gelatinization temperature range. The bimodal endotherm of the *ae wx* starch in the W64A line can also be interpreted as two overlapping peaks, which might have resulted from the presence of two populations of granules or from the existence of two types of molecules within the granules. The higher temperature endothermic peak would be consistent with the melting of longer crystallites formed by the longer A chains and the B1 chains.

TABLE VI
A:B1:(B2 and Longer) Chain Ratios

Starch	A:B1:(B2 + Longer)
Ia5125 line	
wx	52:41:7
ae wx	52:33:15
du wx	52:43:5
W64A line	
wx	52:42:6
ae wx	60:20:20
du wx	47:48:5

Retrogradation. For all the samples, the endothermic transition for the retrograded starch began at a temperature about 20°C lower than that for the gelatinization of the starch granules. The result is consistent with the findings of White et al (1989), who reported that, for waxy maize starch, the T_o of the retrogradation endotherm was about 26°C lower than that of gelatinization. Furthermore, as observed by Shi and Seib (1992), the T_o values are all similar. During storage at 4°C, gelatinized starch molecules reassociate, but in less ordered and hence less stable forms than in the native granular state. This less stable structure melts at a lower temperature than that required to melt the native structure. The endothermic transition temperature for a retrograded starch gel depends upon the storage temperature. It has been shown that, within 5–50°C, the higher the storage temperature, the higher the transition temperature (Nakazawa et al 1985).

After seven days of storage at 4°C, the *ae wx* genotypes in both lines showed broader DSC endotherms than the *wx* and *du wx* genotypes, spanning nearly 50°C compared with 20–25°C for the other genotypes. The breadth of the endotherms for the *ae wx* genotypes may indicate the heterogeneity of the molecular associations in the retrograded starch. As mentioned earlier, the broad gelatinization endotherms for the *ae wx* genotypes might have resulted from two populations of granules or two populations of molecules with different chain length distributions. From gel permeation chromatograms of native starch molecules, Boyer and Liu (1985) showed that several *ae wx* genotypes had an intermediate molecular weight fraction after the major high molecular weight fraction, unlike the *wx* genotypes that had only one major fraction. The intermediate fraction might be similar to the intermediate fraction of amylomaize starch observed by Baba and Arai (1984), who proposed that this material was composed of branched glucans with DP 50 branches. After gelatinization, the extent of reassociation of the amylopectins would be influenced by the length of the unit chains. Molecules with longer unit chains might form longer double helices than the short ones, resulting in a mixture of crystallites of different sizes. The intermediate materials, with longer chains, may therefore form longer double helices. This more ordered structure may account for the high temperature portion of the retrogradation endotherms observed in the *ae wx* genotypes.

For the *wx* and *du wx* genotypes, the starch concentration plays a crucial role in the observed retrogradation thermal behavior. At 10% starch concentration, starch gels of the *wx* genotypes showed no retrogradation. For *du wx* starch gels, the ΔH for the 10% gel was less than one-half that for the 30% gel. This concentration dependence of retrogradation has been reported by Longton and LeGrys (1981) and Zeleznak and Hoseney (1986) for wheat starch. They reported bell-shaped distributions of retrogradation ΔH as a function of starch concentration. Longton and LeGrys (1981) reported that no retrogradation was observed at 4°C if the starch concentration was below 10% or above 80%. Zeleznak and Hoseney (1986) reported a low retrogradation ΔH of about 0.05 cal/g for the 20% wheat starch gel at 25°C. In contrast, the ΔH for the 20% gel reported by Longton and LeGrys (1981) was about 1.6 cal/g, close to the maximum (about 1.9 cal/g) at 50% concentration. The discrepancy might be due to the difference in storage temperatures used (25 vs. 4°C). In the present study, concentration dependence (between 10 and 30%) was not evident for the *ae wx* samples, possibly because of the longer chain length of the amylopectins or the higher proportion of longer B chains in these samples. Gidley et al (1986) reported that the potential for retrogradation of a synthetic amylose increases as DP increases to approximately DP 100. Therefore, the presence of longer DP chains in the *ae wx* samples, together with higher proportion of longer B chains, could still allow the molecules to reassociate at 10% concentration at 4°C.

The argument stated above suggests that the retrogradation process may be influenced by the chain length distribution of amylopectins. The size and shape of the native amylopectins, which was not examined in the present work, could also affect the retrogradation behavior of the gelatinized starches. Boyer and Liu (1985) showed that the sizes of the amylopectins was affected by certain endosperm genotypes. The differences in size or shape of the amylopectins might account for the variation in retrogradation behavior for the *wx* and *du wx* starches at 10% concentration.

The requirements of starch gel stability vary with different applications. The present results suggest that a starch with desirable retrogradation thermal properties might be obtained by appropriate choice of endosperm genotype.

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