# Characterization of the Dominant Mutant Amylose-Extender (*Ae1-5180*) Maize Starch[1]

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**ABSTRACT**

Maize plants homozygous for the dominant amylose extender allele *Ae1-5180* were self-pollinated and crossed onto wild type *Ae* plants, and vice versa, to obtain endosperms with zero to three doses of the *Ae1-5180* allele. Starches from dominant *Ae* mutant kernels were isolated and analyzed with respect to molecular size distribution, amylose contents (iodine affinity), thermal properties (differential scanning calorimetry), and microscopic structure (scanning electron microscopy). Gel-permeation chromatograms of these starches showed greater proportions of amylose and intermediate fractions than those from normal maize starch. The iodine-staining blue value of the amylopectin peak on the profile results revealed longer amylopectin branch-chain lengths than those of normal corn, which was confirmed by enzyme debranching. The structure of the intermediate component and amylopectin was also revealed by gel-permeation chromatography and high-performance anion-exchange chromatography. Greater dosage of the *Ae1-5180* gene did not increase amylose content (≈33%). The differential scanning calorimetry thermograms indicated that all the mutant starches with different dosages had similar thermal properties. The gelatinization onset temperature ($T_o$) range was 65.7–67.0°C and the gelatinization range ($R$) was 54.0–109.7°C. The scanning electron microscope showed that the mutant starches had irregular and round-shaped granules with diameters that ranged from 4 to 18 mm.

Next to cellulose, starch is the most abundant carbohydrate in plants. Starch is an important ingredient in food and has many industrial applications. The versatility of starch utilization (an agent for thickening and gelling, and sizing for paper and textiles) is related to its chemical structure and granular size. The two primary components of starch are amylose and amylopectin. Amylose is essentially a linear molecule containing α(1–4)-linked glucose units with a small number of branches (Greenwood 1964; French 1973, 1975; Hizukuri et al 1981; Takeda and Hizukuri 1987). Amylopectin is a branched molecule with α(1–4)-linked glucose unit linear chains and α(1–6)-linked branch points (Greenwood 1964, French 1973, Lineback 1984). Some studies have shown the existence of a third component in some starches, called the intermediate material (Lansky et al 1949). The amount and structure of the intermediate materials differ with starch type and maturity (Banks and Greenwood 1975, Wang et al 1993). Because of the different functionalities of these components, characterization of the molecular structure of starch is important.

Starch is the major component of maize kernels (72% of the kernel dry weight) (Boyer and Shannon 1987), and it is the primary product obtained in the wet milling of maize. Most of the starch is in the endosperm, but a significant amount is also in the embryo, bran, and tip cap (Watson 1984, Boyer and Shannon 1987). Mutations that alter the levels of starch, amylose and amylopectin proportions, and starch structure in the maize endosperm have been identified (Shannon and Garwood 1984). Starches with new properties and functionalities expanded the possible food and industrial applications and have led to increased research in the role of starch in various applications. The availability of these mutants has also enabled us to study the correlation between the molecular structure and the functional properties of starch.

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One of the mutants, *ae1*, increases the proportion of amylose relative to amylopectin and elongates the branch-chain lengths of amylopectin. Starch obtained from lines homozygous for this mutant can be used to produce tough, edible, and biodegradable films and gels. Recently, a dominant mutant allele of the *ae1* locus was reported (Stinard et al 1993). A dominant mutant allele at the *ae1* locus can be used to accelerate the development of high-amylose inbred or hybrid lines because such a dominant genetic trait, which produces the high-amylose starch, expresses itself in every generation of crossing and thus is readily followed. The recessive *ae* mutant alleles are not as readily followed in a crossing regime (Robertson and Stinard 1991).

In this study, we investigated structures and properties of the dominant mutant amylose-extender (*Ae1-5180*) maize starch. X-ray diffraction was used to reveal the crystalline structure of starch granules and the relative amount of crystalline and amorphous phases within the starch granules (Zobel 1964). Size and shape of the starch granules were recorded by scanning electron microscopy (SEM). Molecular size distributions of the starch were determined by gel-permeation chromatography (GPC). Branch-chain lengths of amylopectin were determined by high-performance anion-exchange chromatography (HP-AEC) and GPC. The intermediate component structure was also determined in the same manner as that of amylopectin. Thermal properties of the starch at the gelatinization and the retrogradation stages were investigated by differential scanning calorimetry (DSC).

## MATERIALS AND METHODS

### Enzyme and Chemicals

Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) was a product of Hayashibara Shoji, Inc. (Okayama, Japan). The specific activity was about 66,000 units per milligram of protein. The enzyme was used directly without further purification. All the chemicals were reagent grade and were used without further treatment.

### Maize Kernel Samples and Chemicals

*Ae* dominant mutant mature maize kernels *(Ae1-5180/Ae1+/Ae1+, Ae1-5180/Ae1-5180/Ae1+, Ae1-5180/Ae1-5180/Ae1-5180)*

and wild type *(Ae1+/Ae1+/Ae+)* were obtained from maize plants grown in 1991 at Iowa State University. Maize plants homozygous for the dominant mutant *amylose-extender1 (ae)* allele *Ae1-5180* were self-pollinated and crossed onto wild type *Ae1* plants, and vice versa, to obtain endosperms with 0, 1, 2, and 3 doses of the *Ae1-5180* allele. The *Ae1-5180* source had been backcrossed for six generations to the inbred A636 and self-pollinated for two generations to achieve homozygosity for *Ae1-5180* in an A636 background (Robertson and Stinard 1991). The inbred A636 was used as the source for the wild type *Ae* allele. Ears were harvested at physiological maturity and then dried for seven days at 39°C. Kernels were shelled off the ears, weighed, and processed for endosperm starch analysis.

### Starch Isolation

Starches were isolated using a slight modification of the method by Badenhuizen (1964). The kernels were soaked in 0.01*M* mercuric chloride solution and then ground and strained through a 30-μm screen. The starch was then isolated by centrifugation and resuspended in 0.1*M* NaCl with 10% volume of toluene. The mixture was stirred for at least 1 hr and allowed to stand until the starch precipitated. The protein-toluene layer was removed, and the procedure was repeated until the starch sediment became clean. After it was washed with distilled water, the starch was dried in a forced-air oven at 40°C.

### Scanning Electron Microscopy

The starch sample was suspended in 100% methanol. The suspension was stirred up, and a drop of the suspension was placed on aluminum tape (nonsticky side) attached to a brass disk. The specimens were coated in a Polaron E5100 sputter coater with gold-palladium (60:40). The mounted specimens were observed using a scanning electron microscope (JEOL JSM-35, Tokyo, Japan) at 10 kV. Micrographs of each starch sample were taken at 1,500× magnification (Jane et al 1994). The diameter of the granular starch was determined by measuring 20 granules.

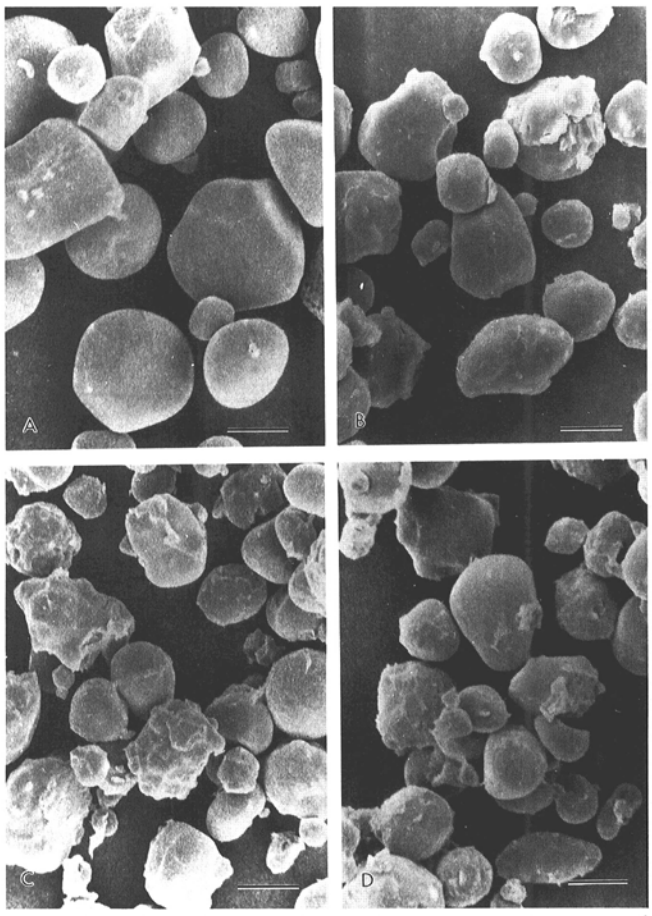


**Fig. 1.** Scanning electron micrographs of maize starch granules (1,500×). **A,** Wild type (*Ae+/Ae+/Ae+*). Dominant mutant *Ae1-5180* with: **B,** one dose (*Ae1-5180/Ae+/Ae+*); **C,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **D,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). Bar = 10 μm.

### X-ray Diffraction Pattern

The x-ray patterns of the starches were obtained with copper, nickel foil-filtered, Kα radiation using a diffractometer (D-500, Siemens, Madison, WI) located at the Engineering Research Institute, Iowa State University. Operation was at 25 μA and 50 kV with a medium resolution and a step-scan mode of 0.05° per step, with a counting time of 2 sec.

### Molecular Size Distribution by GPC

One gram of starch was suspended in 100 ml of 90% dimethyl sulfoxide (DMSO). The suspension was boiled and stirred at 95°C for 2 hr, followed by continuous stirring at room temperature for 24 hr. Fifteen milliliters of the solution (≈150 mg of samples) was precipitated with ≈100 ml of 100% methyl alcohol. The precipitate was separated by centrifugation at 5,000 × *g* for 20 min. The precipitate was then redissolved in 50 ml of hot distilled water with 10 mg of glucose added as a marker. The solution was then boiled and stirred at 95°C for 1 hr. A 5-ml solution containing 15 mg of starch was quickly cooled and injected into a 2.6- × 80-cm column (Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel. Distilled, deionized water containing 10 m*M* NaOH and 50 m*M* NaCl was used to elute the sample in an ascending direction at a 30 ml/hr flow rate. Fractions of 4.8 ml were collected and analyzed (Autoanalyzer II, Technicon Instrument Corp., Elmsford, NY). The total carbohydrate (anthrone-sulfuric acid reaction) and amylose-iodine blue value of the fractions were measured at 630 and 640 nm, respectively. Molecular size distributions were calculated on the basis of total carbohydrate. The blue value was used to identify locations of the amylose and amylopectin in the chromatograms. Chromatograms were replicated at least five times (Jane and Chen 1992).

### Fractionation

Amylose was separated from the mixture of amylopectin and intermediate material according to the methods of Schoch (1942)

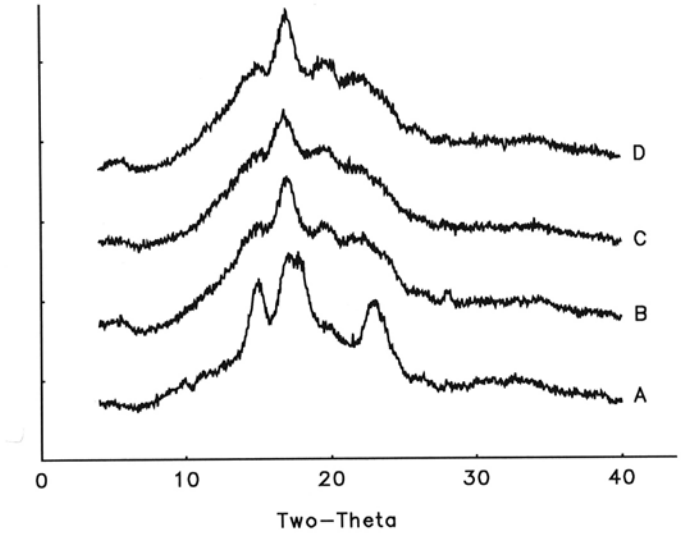


**Fig. 2.** X-ray diffraction patterns of maize starch. **A,** Wild type (*Ae+/Ae+/Ae+*). Dominant mutant *Ae1-5180* with: **B,** one dose (*Ae1-5180/Ae+/Ae+*); **C,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **D,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*).

and Jane and Chen (1992). The fraction containing a mixture of amylopectin and intermediate material was recrystallized five times. The absence of amylose in the mixture of amylopectin and intermediate material was confirmed by GPC.

### Amylose Contents

Amylose contents were determined by measuring iodine affinities of defatted starches using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). The analysis was based on Schoch's method (1964). Determinations were made for the starch samples and mixtures of amylopectin and the intermediate components. Iodine affinities of the samples were replicated four times.

### Branch-Chain Length of Amylopectin and the Intermediate Components

Amylopectin fractions, collected from GPC of the amylopectin and intermediate components mixture (fractionated by Schoch's method), were concentrated by vacuum evaporator at 45°C. An amylopectin solution containing 20 mg of amylopectin in 3.2 ml of distilled water was prepared. An acetate buffer of pH 3.5 (0.1*M*, 0.4 ml) was added and thoroughly mixed before adding *Pseudomonas* isoamylase (900 units). The mixture was incubated in a water bath shaker at 40°C for 48 hr. The enzyme reaction was stopped by heating the mixture in a water bath at 95°C for 30 min. Branch-chain length distribution was analyzed by using a Bio-Gel P-6 gel-permeation column. The chain length of each peak was determined by measuring the reducing value using a modified Park-Johnson method (Park and Johnson 1949, Hizukuri et al 1981, Jane and Chen 1992). Total carbohydrate content was determined using a phenol-sulfuric analysis (Dubois et al 1956) of the peak fractions. Chain length determinations were repeated at least three times.

The intermediate components were also collected from GPC of amylopectin and intermediate component mixtures (fractionated by Schoch's method). Branch-chain length of the intermediate component was analyzed in the same way as amylopectin.

### HP-AEC

The branch-chain length distribution of debranched amylopectins also was analyzed using a Dionex DX-300 system (Sunnyvale, CA) equipped with a pulsed amperometric flow-through cell with a gold-working electrode, a silver-silver chloride reference electrode, and a potentiostat. A debranched sample solution (1.5 mg/ml) was filtered through a 0.45-µm membrane (Supor 450, Gelman Sciences, Ann Arbor, MI). The debranched samples were analyzed according to the method of Wong and Jane (1995). The filtrate (25 µl) was injected and analyzed using a Dionex CarboPac PA1 guard column. The pulsed potentials and durations were: $E_1$ = 0.05 V ($t_1$ = 480 msec), $E_2$ = 0.60 V ($t_2$ = 120 msec), and $E_3$ = -0.60 V ($t_3$ = 60 msec) at range 2 (sampling periods, 200 msec). The eluents A and B were 150 m*M* sodium hydroxide solution and 150 m*M* sodium hydroxide in 500 m*M* sodium nitrate solution, respectively. The solutions were degassed with

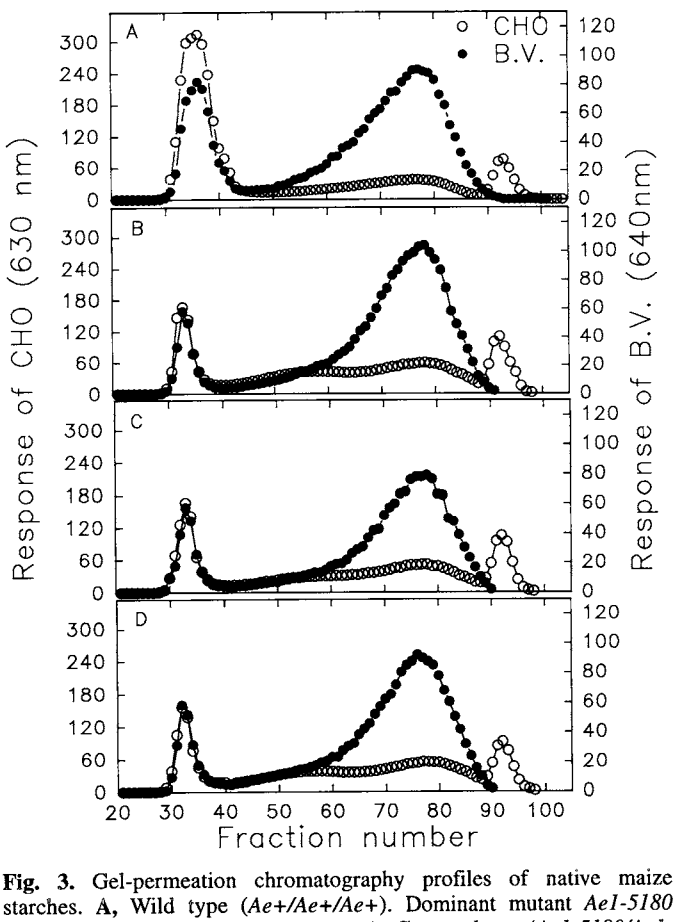


**Fig. 3.** Gel-permeation chromatography profiles of native maize starches. **A,** Wild type (*Ae+/Ae+/Ae+*). Dominant mutant *Ae1-5180* with: **B,** one dose (*Ae1-5180/Ae+/Ae+*); **C,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **D,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). Sepharose CL-2B column (2.6 i.d. × 90 cm) eluted with 50 m*M* NaCl aqueous solution and 10 m*M* NaOH. Flow rate was 0.5 ml/min. o—o = Fractions (4.8 ml) analyzed for total carbohydrate (anthrone-sulfuric acid procedure). ●—● = Blue value (amylose-iodine complex). Glucose was used as the marker.

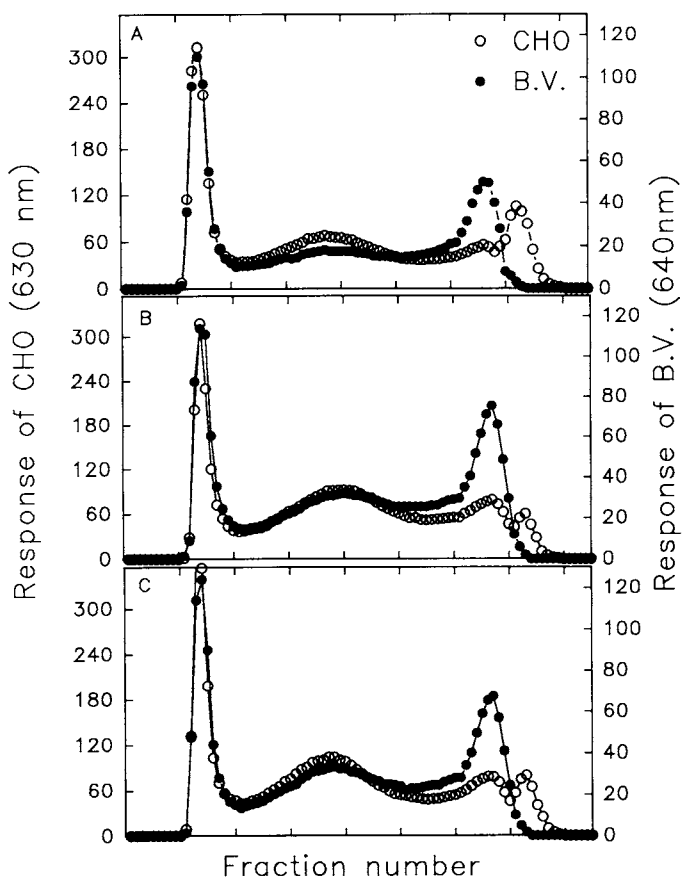


**Fig. 4.** Gel-permeation chromatography profiles of mixed amylopectin and intermediate components. Dominant mutant *Ae1-5180* maize starch with: **A,** one dose (*Ae1-5180/Ae+/Ae+*); **B,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **C,** three doses(*Ae1-5180/Ae1-5180/Ae1-5180*). Sepharose CL-2B column (2.6 i.d. × 90 cm) eluted with 50 m*M* NaCl aqueous solution and 10 m*M* NaOH. Flow rate was 0.5 ml/min. o—o = Fractions (4.8 ml) analyzed for total carbohydrate (anthrone-sulfuric acid procedure). ●—● = Blue value (amylose-iodine complex). Glucose was used as the marker.

helium by a Dionex degas module. The eluent gradient, operated with a flow rate at 1 ml/min, was: 0–5 min, 94% of eluent A and 6% of eluent B; 5–10 min, linear gradient to 8% eluent B; 10–30 min, linear gradient to 13% eluent B; 30–80 min, linear gradient to 20% eluent B; 80–100 min, linear gradient to 25% eluent B. HP-AEC was repeated six times.

**TABLE I**
**Percentage of Amylose Contents and Iodine Affinity in Starches**

| Type | Apparent Amylose Content (%)[a] (Iodine Affinity[b]) | | Real Amylose Content (%) |
|---|---|---|---|
| | Defatted Starch | Mixture of Amylopectin and Intermediate Components | |
| *Ae+/Ae+/Ae+* | 29.0 ± 0.4 (5.5 ± 0.1) | 6.4 ± 0.2 (1.2 ± 0.1) | 24.1 ± 0.6 |
| *Ae1-5180/Ae+/Ae+* | 58.3 ± 0.5 (11.1 ± 0.1) | 34.7 ± 0.4 (6.6 ± 0.1) | 36.3 ± 0.9 |
| *Ae1-5180/Ae1-5180/Ae+* | 53.8 ± 1.0 (10.2 ± 0.2) | 34.5 ± 0.2 (6.6 ± 0.1) | 29.0 ± 1.2 |
| *Ae1-5180/Ae1-5180/Ae1-5180* | 55.5 ± 0.9 (10.6 ± 0.2) | 33.5 ± 0.9 (6.4 ± 0.2) | 33.3 ± 1.8 |

[a] Amylose contents were determined by iodine potentiometric titration and were calculated by dividing iodine affinity by a factor of 0.19.
[b] Iodine affinities were averaged from at least two starch samples with at least four replicates of each sample.

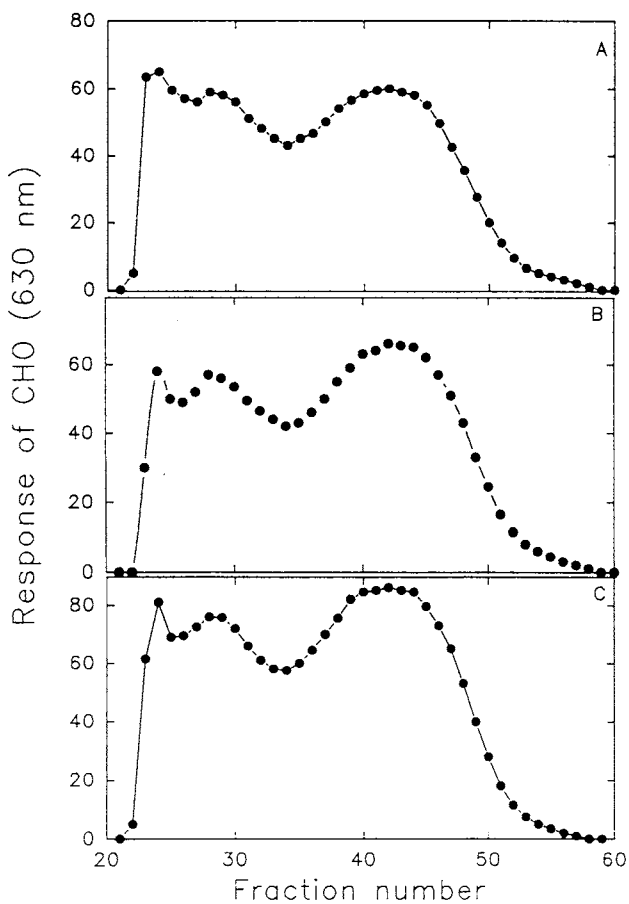


**Fig. 5.** Gel-permeation chromatography profiles of debranched amylopectins. Dominant mutant *Ae1-5180* maize starch with: **A,** one dose (*Ae1-5180/Ae+/Ae+*); **B,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **C,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). Bio-Gel P-6 column (1.5 i.d. × 80 cm) eluted with distilled deionized water. ●—● = Fractions (2.3 ml) analyzed for total carbohydrate (anthrone-sulfuric acid procedure).

## Thermal Properties

Thermal properties of starches were determined according to a slightly modified procedure by Wang et al (1992). The DSC studies were performed using a Perkin-Elmer DSC-7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). Starch samples (≈10.0 mg) were weighed into stainless steel pans (Perkin Elmer) using a microbalance (AE240, Mettler, Hightstown, NJ). Distilled water (≈21.0 mg) was added. The pan was hermetically press-sealed and allowed to equilibrate for ≈12 hr before analysis. The samples were heated at 10°C/min over a temperature range of 25–150°C. Indium was used as the reference standard. Enthalpy ($\Delta H$), onset temperatures ($T_o$), peak temperatures ($T_p$), and gelatinization ranges ($R$) were computed automatically. The data were calculated from at least three tests of each genotype. The same method was used for retrogradation of the gelatinized samples kept at 4°C for seven days.

Isolation of this starch was difficult because of tightly bound protein on the surface of starch granules. Starch contents in the kernels were ≈43–51%, and the starch yields were ≈30–40%, equivalent to recovery rates of 65–80%. The contamination of protein would be high if the starch were isolated extensively.

Scanning electron micrographs of starch granules, isolated from the wild type (*Ae+/Ae+/Ae+*) and *Ae1-5180* dominant mutant starches (*Ae1-5180/Ae+/Ae+*, *Ae1-5180/Ae1-5180/Ae+* and *Ae1-5180/Ae1-5180/Ae1-5180*), are shown in Figure 1a–d. The diameter of the granular wild type (*Ae+/Ae+/Ae+*) starch ranged from 5 to 20 μm, and the diameter of the *Ae1-5180* dominant mutant starch, with one to three doses of dominant mutant genes, ranged from 4 to 18 μm. Granular shapes of the wild type starch were similar to those of normal maize starch, which are polygonal (Banks et al 1974, Wang 1993, Jane et al 1994). The granular shapes of the *Ae1-5180* dominant mutant starches with one to three doses were all similar, typically spherical or oval, with some containing budlike attachments, but none showed a polygonal shape. The shapes of the *Ae1-5180* dominant mutant starches were similar to those of the high-amylose maize starch (*ae*) (Banks et al 1974, Wang 1993), but *Ae1-5180* dominant mutant did not have long rodlike granules.

X-ray diffraction of the wild type starch (*Ae+/Ae+/Ae+*) showed that the A pattern (Fig. 2a) was the same as that of normal maize starch (Zobel 1964). The *Ae1-5180* dominant mutant starches (*Ae1-5180/Ae+/Ae+*, *Ae1-5180/Ae1-5180/Ae+*, *Ae1-5180/Ae1-5180/Ae1-5180*) showed that the B pattern (Fig. 2b–d) was the same as that of high-amylose maize starch (*ae*). The x-ray patterns also indicated low degrees of crystallinity in the *Ae1-5180* dominant mutant starch granules. These results were consistent with x-ray data of the high-amylose starches (French 1984). The difference could be because amylopectin is the main component responsible for the crystallinity of starch.

**TABLE II**
**Summary of Amylopectin Branch-Chain Length Distribution[a]**

| Type | Chain Length (glucose units) | | Ratio of Total Carbohydrate Contents (long chains: short chains) |
|---|---|---|---|
| | Long Chains[b] | Short Chains[c] | |
| *Ae+/Ae+/Ae+* | 42.3 ± 0.9 | 13.9 ± 2.1 | 1:3.0 |
| *Ae1-5180/Ae+/Ae+* | 45.7 ± 2.8 | 19.0 ± 0.4 | 1:1.8 |
| *Ae1-5180/Ae1-5180 /Ae+* | 44.5 ± 3.5 | 19.1 ± 0.7 | 1:1.7 |
| *Ae1-5180/Ae1-5180 /Ae1-5180* | 46.6 ± 3.3 | 19.1 ± 0.6 | 1:1.6 |

[a] Amylopectin branch-chain lengths were averages of at least two starch samples with at least three replicates of each sample.
[b] Peak chain length of the long chain (B2) of amylopectin.
[c] Peak chain length of the short B and A chains of amylopectin.

Crystalline domains of starch granules are composed of A-chains as well as the exterior parts of B-chains of amylopectin.

Molecular size distributions of the wild type starch and *Ae1-5180* dominant mutant starches were determined by GPC (Fig. 3a-d. The first peak in the chromatograms eluted out early at the void volume and corresponded to amylopectin. The second major peak, displaying a great blue value peak, corresponded to that of amylose. The last peak was glucose added as a marker for the profile. In the *Ae1-5180* dominant starch there was another broad peak between the amylopectin and amylose peak, an intermediate fraction. The chromatogram of the wild type starch showed an elution profile similar to that of the native normal maize starch (Wang et al 1993). The chromatograms of the *Ae1-5180* dominant mutant starch with different doses of dominant mutant gene (*Ae1-5180/Ae+/Ae+*, *Ae1-5180/Ae1-5180/Ae+*, and *Ae1-5180/Ae1-5180/Ae1-5180*) showed similar profiles. The dominant mutant starch chromatograms showed greater total carbohydrate peaks of the intermediate and amylose fractions, but the blue value of the amylose fractions was similar or slightly lower than that of the wild type. The blue value of the amylopectin peak was comparably higher (relative to the amylopectin carbohydrate peak) than that of the wild type, indicating long branch-chain lengths of the amylopectin. The blue value and total carbohydrate ratio of the intermediate fractions was similar to that of the amylopectin. Evidently, the intermediate components were smaller sized amylopectin molecules. The elution profiles of the *Ae1-5180* dominant mutant starches resembled that of *ae btl* mutant maize starch (Wang et al 1993), but differ from that of high amylose (*ae*) starch (Wang et al 1993).

Butanol-soluble fraction obtained by the Schoch method (containing both amylopectin and intermediate component) were analyzed by GPC (Fig. 4a-c). The chromatograms showed the high ratio of the blue value to the total carbohydrate, which indicated long branch chain length in both the intermediate component and amylopectin molecules. It also showed that the mixtures are free from contamination of amylose. The peak that appeared before the glucose marker is attributed to maltodextrin, which cannot be removed by precipitation with *n*-butyl alcohol. Amylopectin-to-intermediate component weight ratio is 1:2.6.

Amylose contents of the starches were analyzed by measuring iodine affinity. The amylose content and iodine affinity are shown in Table I. The iodine affinity of the wild type starch (*Ae+/Ae+/Ae+*) was similar to that of the normal maize starch. Iodine affinities of the *Ae1-5180* dominant mutant starches were similar to that of the high amylose V starch, which contains ≈55% amylose, but the high amylose V starch did not contain as high an amount of the intermediate component (Jane and Chen 1992) as the *Ae1-5180* dominant mutant maize starch did. The real amylose contents of *Ae1-5180* were calculated by the equation:

$$C = (IA_S - IA_{AP+IC}) / [0.19 - (IA_{AP+IC} / 100)]$$

where $C$ is the percentage of the real amylose content; $IA_S$ is the iodine affinity of the whole defatted starch; $IA_{AP+IC}$ is the iodine affinity of the amylopectin and intermediate component mixture. The high iodine affinity of the amylopectin and intermediate component mixture also confirmed the presence of long branch chains that could interact with iodine.

Amylopectin structure was investigated by determination of branch-chain length. GPC of isoamylase-debranched amylopectin samples displayed three major peaks (Fig. 5). The first peak, eluted at the void volume of the column, contained some very long B chains (which could be B3 or longer) (Hizukuri 1986). The second peak corresponded to the B2 chains of amylopectin (long chains). The last peak contained A chains and short B chains of amylopectin (short chains). The fractions collected from the second and third peaks were analyzed for their peak chain lengths. The ratio of the long chain to short chain contents and the peak chain lengths of the long and short chains are shown in Table II. These results confirmed those of GPC, iodine affinity, and X-ray diffraction determinations, which indicated longer chain length in the mutant amylopectin than in the wild type starch. The amylopectin chain length for wild type starch was similar to that of normal maize. The chain length of the *Ae1-5180* dominant mutant for different doses of dominant mutant genes was shorter than the branch-chain lengths of the high-amylose (*ae*) V and VII starches (Wang et al 1993, Chen and Jane 1995).

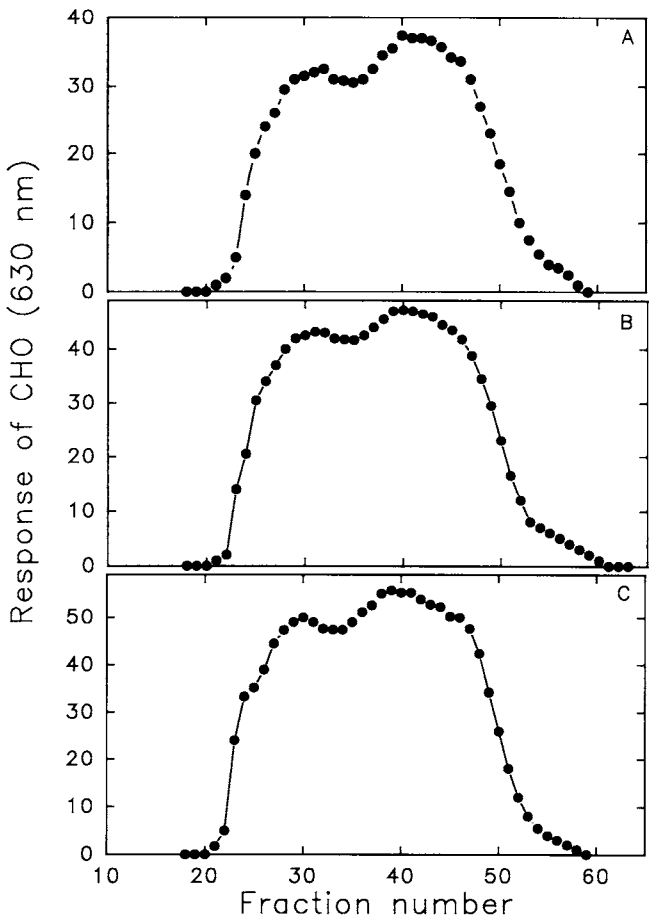


**Fig. 6.** Gel-permeation chromatography profiles of debranched intermediate components. Dominant mutant *Ae1-5180* maize starch with: **A,** one dose (*Ae1-5180/Ae+/Ae+*); **B,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **C,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*). Bio-Gel P-6 column (1.5 i.d. × 80 cm) eluted with distilled deionized water. ●—● = Fractions (2.3 ml) analyzed for total carbohydrate (anthrone-sulfuric acid procedure).

**TABLE III**
**Summary of Intermediate-Component Branch-Chain Length Distribution[a]**

| Type | Chain Length (glucose units) | | Ratio of Total Carbohydrate Contents (long chains: short chains) |
|---|---|---|---|
| | Long Chains[b] | Short Chains[c] | |
| *Ae1-5180/Ae+/Ae+* | 51.1 ± 4.0 | 21.8 ± 0.8 | 1 : 1.6 |
| *Ae1-5180/Ae1-5180/Ae+* | 51.6 ± 0.5 | 20.2 ± 1.7 | 1 : 1.7 |
| *Ae1-5180/Ae1-5180/Ae1-5180* | 52.9 ± 4.4 | 21.8 ± 0.9 | 1 : 1.7 |

[a] The intermediate-component branch-chain-lengths were averages of at least two starch samples with at least three replications of each sample.
[b] Peak chain-length of the long chain (B2) of intermediate components.
[c] Peak chain-length of the short B and A chains of intermediate components.

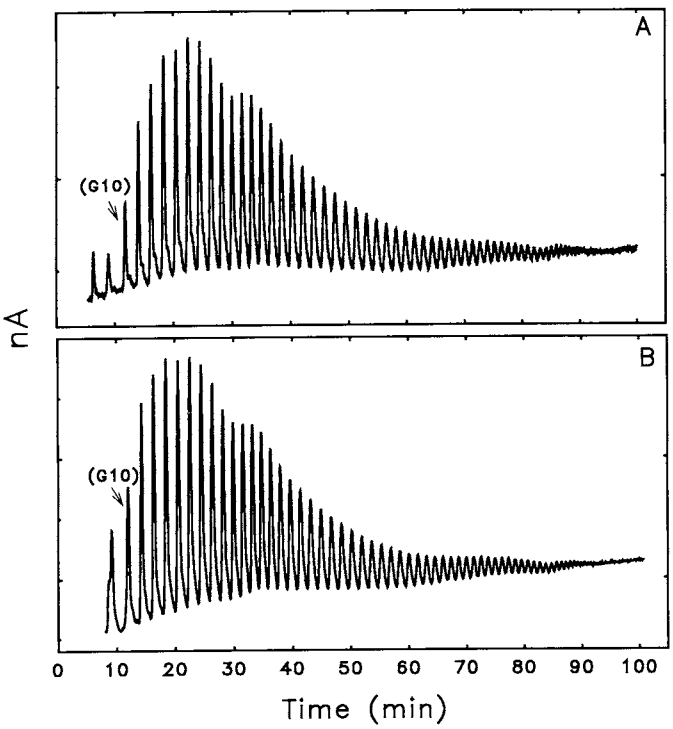


**Fig. 7.** High-performance anion-exchange chromatography of maize starch granules. **A,** *Ae1-5180/Ae1+/Ae1+* dominant mutant debranched amylopectin. **B,** *Ae1-5180/Ae1+/Ae1+* dominant mutant debranched intermediate component. Pulse amperometric detection with gold electrode.

Intermediate component structure was investigated using the same procedure as used for amylopectin. GPC of debranched intermediate component (Fig. 6) displayed two major peaks, as compared with the chromatograms of debranched amylopectin, which contained three peaks. The intermediate component consisted of long chains (B2) (the first peak) and short B and A chains (the second peak). There were no B3 and longer branchchains. The peak chain length and the ratio of long chain to short chain total carbohydrate contents are shown in Table III. These results indicated that the intermediate component did not have B3 and longer chains but had longer B2 and short branch chains.

HP-AEC chromatograms of the debranched *Ae1-5180* dominant mutant amylopectin is shown in Figure 7a, and HP-AEC chromatograms of debranched *Ae1-5180* dominant mutant intermediate component is shown in Figure 7b. The profiles were limited to about DP 65 because of the signal-to-noise ratio. The chromatograms of the *Ae1-5180* dominant mutant amylopectin and the intermediate component with different doses of dominant genes showed similar bimodal distributions. The HP-AEC results were consistent with those of the gel-permeation chromatograms showing that the different doses of the dominant mutant genes did not differ from the amylopectin and intermediate component branch-chain length. It also indicated that both amylopectin and the intermediate component had a similar molecular structure, except the intermediate component had more very short chains (DP 9–14) than amylopectin, and amylopectin had more chains of DP 16–30 than the intermediate component (calculated from the peak area of the normalized chromatograms).

Gelatinization thermograms of the *Ae1-5180* dominant mutant starches determined by DSC showed two peaks (Fig. 8b–d) that were different from that of the wild type starch (Fig. 8a). The second peaks in the *Ae1-5180* dominant mutant starch were referred to as amylose lipid complex. The thermograms of the *Ae1-5180* dominant starches also were different from those of high-amylose starches (*ae*). The gelatinization temperature and

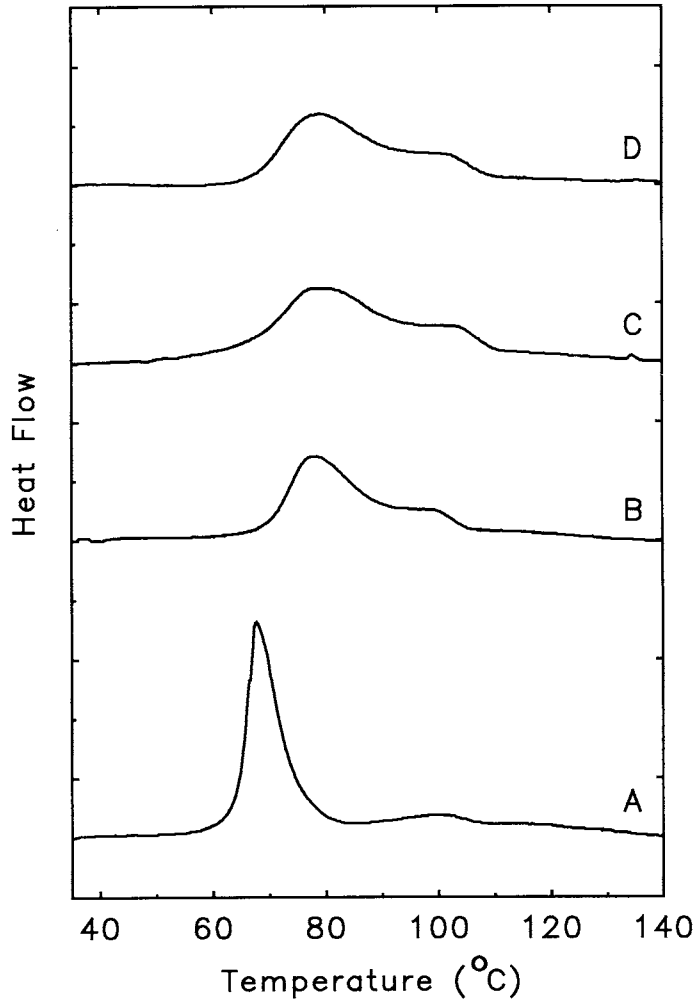


**Fig. 8.** Differential scanning calorimetry thermograms of native maize starches. **A,** Wild type (*Ae+/Ae+/Ae+*). Dominant mutant *Ae1-5180* with: **B,** one dose (*Ae1-5180/Ae+/Ae+*); **C,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **D,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*).

enthalpy changes are reported in Table IV. The gelatinization temperatures of *Ae1-5180* with different doses of mutant gene were similar, and they were all higher than that of the wild type starch ($T_p$ 69.5). The $T_o$and $T_p$for gelatinization of the *Ae1-5180* dominant maize starches were lower than those of *ae*. The $\Delta H$ of the wild type starch (14.7 ± 1.1 J/g) was greater than that of the dominant mutant starches (≈13.7 J/g).

Retrogradation of the starch sample after storage at 4°C for seven days was analyzed by DSC using the same parameters as for gelatinization. The thermogram (Fig. 9a) of the retrograded wild type starch (*Ae+/Ae+/Ae+*) was similar to that of the normal starch. The thermograms of the retrograded *Ae1-5180* dominant mutant starches showed broad peaks (Fig. 9b–d). The thermal transition temperatures and enthalpy changes for the retrograded starches are shown in Table V. Thermal transition ranges of the retrograded starches were broader than those of the gelatinization of native starches. The enthalpy change of the retrograded wild type starch was lower than those of the mutant starches, which could be attributed to the greater tendency of reassociation of the longer branch chains of the *Ae1-5180* dominant mutant starches. The thermograms of the high-amylose 50 and 70% starches showed very broad peaks, and it is difficult to identify the real peaks. Thermograms of native and retrograded starches of all the varieties showed a peak at ≈100°C, which was attributed to the amylose-lipid complex. The amylose-lipid complex peak was confirmed by rescanning, which showed the peak occurring at the same position. The enthalpies of the dominant mutant *Ae1-5180*

TABLE IV
Thermal Properties of Starch Gelatinization Determined by Differential Scanning Calorimetry[a]

| Type | $T_o$[b] (°C) | $T_p$[c] (°C) | $R$ (°C) | $\Delta H_{S+A-L}$[d] (J/g) | $\Delta H_{A-L}$[e] (J/g) | $\Delta H_S$[f] (J/g) |
|---|---|---|---|---|---|---|
| *Ae+/Ae+/Ae+* | 65.7 ± 1.5 | 69.5 ± 1.9 | 56.1 ± 0.5 to 85.0 ± 2.6[g] | 15.1 ± 1.1 | 0.4 ± 0.1 | 14.7 ± 1.1 |
| *Ae1-5180/Ae+/Ae+* | 65.7 ± 1.8 | 78.1 ± 1.0 | 58.3 ± 4.4 to 109.2 ± 1.3[h] | 14.6 ± 0.8 | 1.0 ± 0.1 | 13.5 ± 0.8 |
| *Ae1-5180/Ae1-5180/Ae+* | 66.7 ± 0.8 | 78.9 ± 1.7 | 54.0 ± 1.3 to 109.7 ± 1.4[h] | 14.6 ± 0.7 | 0.7 ± 0.2 | 13.9 ± 0.9 |
| *Ae1-5180/Ae1-5180/Ae1-5180* | 67.0 ± 3.3 | 78.9 ± 2.1 | 57.3 ± 3.4 to 108.8 ± 1.3[h] | 14.4 ± 1.2 | 0.6 ± 0.2 | 13.8 ± 1.4 |
| High amylose 50% | 67.3 ± 1.2 | 75.0 ± 1.7 | 62.5 ± 0.5 to 109.5 ± 1.3[h] | 17.1 ± 0.4 | 1.0 ± 0.1 | 16.1 ± 0.4 |
| High amylose 70% | 69.3 ± 0.4 | 75.3 ± 0.7 | 65.7 ± 0.9 to 109.4 ± 0.5[h] | 12.7 ± 0.8 | 1.1 ± 0.1 | 11.6 ± 0.8 |

[a] The values are averages of at least three starch samples with at least three replicates of each sample.
[b] Onset temperature.
[c] Peak temperature.
[d] Enthalpy of starch gelatinization and melting of amylose-lipid complex.
[e] Enthalpy of melting amylose-lipid complex.
[f] Gelatinization enthalpy of starch ($\Delta H_{S+A-L} - \Delta H_{A-L}$).
[g] Gelatinization range
[h] Gelatinization and melting amylose-lipid complex range.

TABLE V
Thermal Properties of Retrograded Starch Determined by Differential Scanning Calorimetry[a]

| Type | $T_o$[b] (°C) | $T_p$[c] (°C) | $R$ (°C) | $\Delta H_{S+A-L}$[d] (J/g) | $\Delta H_{A-L}$[e] (J/g) | $\Delta H_S$[f] (J/g) |
|---|---|---|---|---|---|---|
| *Ae+/Ae+/Ae+* | 41.1 ± 0.3 | 51.5 ± 0.1 | 36.9 ± 1.9 to 67.6 ± 2.3[g] | 7.8 ± 1.0 | 0.8 ± 0.1 | 7.0 ± 1.1 |
| *Ae1-5180/Ae+/Ae+* | 42.8 ± 2.0 | 59.9 ± 1.0 | 42.3 ± 1.7 to 106.8 ± 1.5[h] | 8.8 ± 0.4 | 1.0 ± 0.1 | 7.8 ± 0.4 |
| *Ae1-5180/Ae1-5180/Ae+* | 43.7 ± 1.6 | 64.2 ± 4.2 | 43.0 ± 2.6 to 107.9 ± 1.2[h] | 9.2 ± 0.8 | 0.9 ± 0.1 | 8.3 ± 0.8 |
| *Ae1-5180/Ae1-5180/Ae1-5180* | 46.9 ± 2.9 | 69.4 ± 1.0 | 44.5 ± 2.6 to 109.3 ± 1.8[h] | 9.2 ± 1.4 | 1.0 ± 0.1 | 8.2 ± 1.5 |
| High amylose 50% | 40.0 ± 1.6 | NA[i] | 36.1 ± 0.4 to 111.1 ± 2.6[h] | 10.1 ± 1.0 | 1.2 ± 0.1 | 9.0 ± 1.1 |
| High amylose 70% | 43.4 ± 0.3 | NA | 39.5 ± 0.4 to 110.1 ± 1.2[h] | 6.8 ± 1.1 | 1.2 ± 0.1 | 5.6 ± 1.1 |

[a] The values are averages of at least three starch samples with at least three replicates of each sample.
[b] Onset temperature.
[c] Peak temperature.
[d] Enthalpy of melting retrograded starch and melting of amylose-lipid complex.
[e] Enthalpy of melting amylose-lipid complex.
[f] Enthalpy of melting retrograded starch ($\Delta H_{S+A-L} - \Delta H_{A-L}$).
[g] Melting range
[h] Melting and amylose-lipid complex range.
[i] Not available.

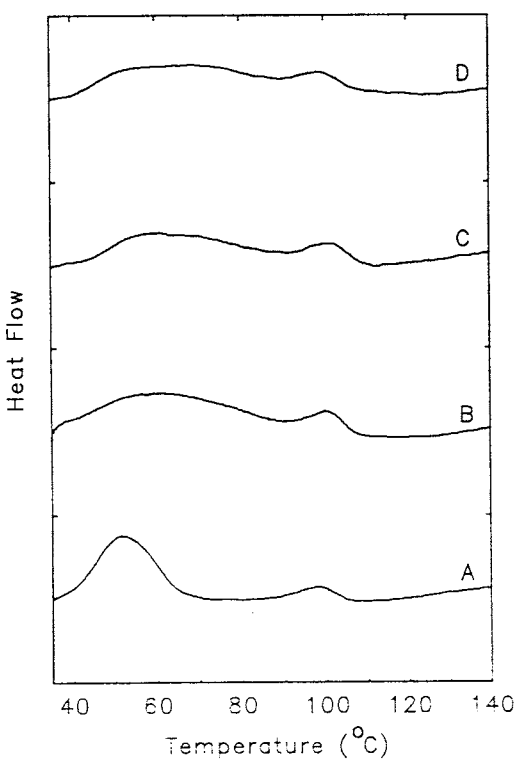


**Fig. 9.** Differential scanning calorimetry thermograms of retrograded maize starches. **A,** Wild type (*Ae+/Ae+/Ae+*). Dominant mutant *Ae1-5180* with: **B,** one dose (*Ae1-5180/Ae+/Ae+*); **C,** two doses (*Ae1-5180/Ae1-5180/Ae+*); **D,** three doses (*Ae1-5180/Ae1-5180/Ae1-5180*).

amylose-lipid complex (0.9 ± 0.3 J/g) were higher than that of wild type amylose lipid complex (0.6 ± 0.1 J/g). This indicated that the dominant mutant *Ae1-5180* starch contained more amylose-lipid complex than did the wild type starch.

## SUMMARY

The characteristics of the *Ae1-5180* dominant mutant maize starches with different doses of genes did not show any significant difference. SEM showed that some protein attached to the surface of starch granules. Results from SEM, x-ray diffraction patterns, GPC, structure of amylopectin, HP-AEC, and thermal properties indicated that *Ae1-5180* dominant mutant maize starches were different from the common high-amylose maize starch isolated from the recessive (*ae*) mutants. The analysis of amylopectin and intermediate component suggested that the amylopectin branch-chain length was shorter than that of the intermediate component. The real amylose contents of *Ae1-5180* dominant mutant maize starch was less than that of high-amylose 50% starch and high-amylose 70% starch, but the *Ae1-5180* dominant mutant starch contained very high amounts of intermediate components.

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