

Amylose and Amylopectin Interact in Retrogradation of Dispersed High-Amylose Starches

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ABSTRACT

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Retrogradation of three high-amylose starches (HAS: *ae du*, *ae V*, and *ae VII*) and common corn starch (CCS) was examined by dynamic oscillatory rheometry (7.5% [w/w] starch in 20% [v/v] dimethyl sulfoxide [DMSO]), differential scanning calorimetry (DSC; 30% [w/w] starch in water), and turbidity (0.5% [w/w] starch in 20% [v/v] DMSO). Nongranular lipid-free starch and starch fractions (amylose [AM], amylopectin [AP], and intermediate material [IM]) were studied. Gels were prepared by dispersing starches or fractions in 90% DMSO and diluting with water, followed by storage for seven days at 4°C. For AM from each starch, the elastic modulus (G') was similar when heated from 6 to 70°C. The G' of HAS AP gels at 6°C was higher than for CCS AP gels. For nongranular

CCS and *ae du* gels, G' dropped dramatically ($\approx 100\times$) when heated from 6 to 70°C, less ($\approx 10\times$) for *ae V* gels, and least ($\approx 5\times$) for *ae VII* gels. By DSC, each AM endotherm had a peak temperature of $\approx 140^\circ\text{C}$, whereas all AP endotherms were complete before 120°C. Endotherms $>120^\circ\text{C}$ were not observed for any nongranular starch despite the high AM content of some starches. After cooling starch suspensions from room temperature to 5°C and subsequent rewarming to room temperature, each AM and the *ae VII* nongranular starch remained highly turbid. Each AP and the remaining nongranular starches lost turbidity during rewarming. Our work suggests that branched molecules of CCS and HAS influence gel properties of nongranular starches by inhibiting or altering AM-AM interactions.

Retrogradation is the process by which starch returns to a more ordered state after gelatinization (Atwell et al 1988). During retrogradation, amylose may form double-helical associations of 40–70 glucose units (Jane and Robyt 1984, Leloup et al 1992, Liu et al 1997), whereas amylopectin forms shorter double helices than amylose due to restrictions imposed by the branching structure of the molecules and the chain lengths of the branches. Double helices may associate and organize into crystallites (Miles et al 1985, Ring et al 1987), and gelation results under appropriate conditions. Nuclear magnetic resonance (NMR) (Gidley 1989, Gidley and Bulpin 1989), X-ray diffraction (Miles et al 1984, I'Anson et al 1988), and rheological (Gidley 1989) studies of amylose dispersions indicate that amylose gels are composed of a network of double-helical, semi-crystalline junction zones separated by amorphous regions.

Gudmundsson (1994) pointed out that starch gels from normal starch, as well as gels from amylose of normal starch, are thermally reversible at 100°C, in contrast to amylose gels. Nevertheless, Gudmundsson (1994) suggested that interaction between amylose and amylopectin would be limited in normal starch if granule integrity remains. Studies of the mechanism of amylopectin gelation indicate that amylopectin gels may develop through the formation of short intermolecular aggregates (Miles et al 1985, Ring et al 1987). Ring et al (1987) observed a loss of turbidity in 20% amylopectin gels between 40 and 60°C, a temperature range that coincided with a differential scanning calorimetry (DSC) endotherm. After acid hydrolysis of gels, debranching of residue by pullulanase, and subsequent size-exclusion chromatography, a single peak with a maximum degree of polymerization (DP) at 15 and a shoulder at DP 30–40 was observed, indicating the portion of the gel structure resistant to acid was composed primarily of short (DP 15) chains (Ring et al 1987). Because the β -limit dextrans of amylopectin failed to form gels at similar concentrations and failed to develop turbidity when held at 1°C for one month, Ring et al (1987) suggested that the small chains involved in the gel network were the exterior chains of the amylopectin molecules. Based on the turbidity and rheological behavior of aqueous waxy maize starch dispersions at starch concentrations between 3 and 10.5%, Cameron et al (1994) suggested that the formation of infrequent intermolecular double helices between exter-

nal chains of molecules, without formation of crystallinity, also contributes to the network structure of amylopectin gels.

Jane and Chen (1992) fractionated potato, normal maize, and high-amylose maize starch and prepared 8% (w/w) starch gels by co-dispersing the fractions (amylose:amylopectin, 1:4, w/w) in 0.5M KOH and neutralizing the dispersions to effect gelation. The mixed gels containing amylopectin with longer branches formed stronger gels than those containing amylopectin with shorter branches, indicating that the amylopectin with longer chains did not simply act as a filler within an amylose gel matrix. Parovuori et al (1997) prepared mixed gels containing either 25 or 50% amylose, with the remainder being either waxy maize starch or a dextrin (classified as large, medium, or small molecular weight by size-exclusion chromatography) prepared by limited α -amylase digestion. The mixed gels containing either 25 or 50% amylose and medium-sized dextrans produced the weakest gels, and for gels containing the same dextrin, gels containing 25% amylose formed weaker gels than gels containing 50% amylose. These observations show that both amylose content and amylopectin size affect the properties of mixed gels.

Boltz and Thompson (1999) demonstrated that the retrogradation enthalpy of granular and nongranular high-amylose starches depended on the temperature to which starches were heated. For starches heated to 120 or 140°C, when subsequently reheated, an endotherm $>120^\circ\text{C}$ was observed and attributed to amylose. This result showed that amylose could associate independently of branched molecules. Sievert and Pomeranz (1989, 1990) also observed endotherms $>120^\circ\text{C}$ after starches were autoclaved between 121 and 148°C. However, after Boltz and Thompson (1999) initially heated starches to 160 or 180°C, they did not observe endotherms $>120^\circ\text{C}$ when reheated, suggesting the ability of amylose to associate independently had been lost.

Starches from amylose-extender (*ae*), dull (*du*), and sugary-2 (*su2*) and from *ae du* and *ae su2* maize endosperm have an apparent amylose content higher than that of normal maize starch (Shannon and Garwood 1984). Previous work in our laboratory has shown that *ae du* starch behaves differently than *ae* starches, but that *ae su2* starch behaves similarly to *ae* starches (Ferrone 1996). The *ae* mutation results in a loss of starch branching enzyme IIb activity (Boyer and Preiss 1978). Recent evidence suggests that the *du* mutation results in loss of starch synthase II activity (Gao et al 1998) and affects the activity of at least one other starch biosynthetic enzyme (Boyer and Preiss 1981). In addition to the high apparent amylose content, *ae* starches contain branched molecules that have a higher proportion of longer chains (DP >30) than the amylopectin of common corn starch (Takeda et al 1993, Klucinec and Thompson

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1998), and the branched molecules are partially separated by Sepharose CL-2B chromatography (Baba et al 1982, Baba and Arai 1984, Wang et al 1993b, Klucinec and Thompson 1998). The *ae du* starches contain branched molecules with chain profiles more closely resembling amylopectin of common corn starch (Ferrone 1996) and are partially separated by Sepharose CL-2B chromatography (Wang et al 1993a,b).

Kalichevsky and Ring (1987) showed that 6% dispersions of normal amylose and amylopectin behave as incompatible polymers at 80°C. German et al (1992), working with 1.5% amylose and 1.9% amylopectin at 85°C, made a similar observation. Nevertheless, Jane and Chen (1992), Boltz and Thompson (1999), and Parovuori et al (1997) demonstrated that retrogradation or gelation of amylose-amylopectin mixtures cannot be explained based on simple combinations of the retrogradation and gelation abilities of each component of the mixture. The contributions of amylose and amylopectin to gels of different high-amylose maize starches have not been studied.

In a previous study (Klucinec and Thompson 1998), three lipid-free high-amylose starches (HAS) were each separated into three fractions, designated amylose (AM), amylopectin (AP), and intermediate material (IM), by a multistep, differential alcohol fractionation procedure. The purpose of the current study was to understand the contributions of these starch fractions to the gelation of the corresponding dispersed, lipid-free HAS and normal starch to gain a better understanding of gelation of high-amylose starches relative to normal starch and differences in gelation among high-amylose maize starches. Rheological behavior of gels from dispersed, lipid-free normal and high-amylose starches and of the AM and AP fractions from the starches (Klucinec and Thompson 1998) was examined. Turbidity development and thermal behavior of AM, AP, and IM were studied to gain insight into the nature of the gels formed.

MATERIALS AND METHODS

Starch

Maize starches and reagents used were those described previously by Klucinec and Thompson (1998). Two commercial *ae* genotype starches (Hylon V and Hylon VII) and a commercial normal maize starch (Melojel) were obtained from National Starch and Chemical Co. (Bridgewater, NJ). A commercial *ae du* genotype starch

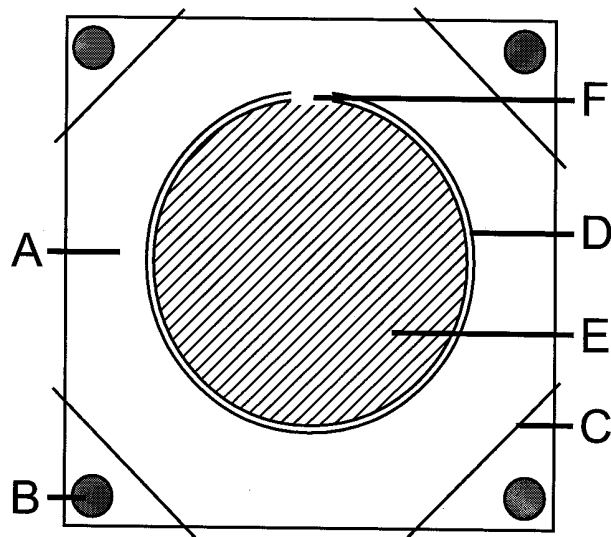


Fig. 1. Gel casting apparatus for dynamic oscillatory rheometry. A, Plexiglas plates; B, 2.4-mm spacers; C, binder clip contact point; D, flexible tubing; E, well for starch paste; F, gap for sample loading.

(Amerimaize 2300) was obtained from American Maize Products Co. (now Cerestar USA, Hammond, IN). The HAS will be referred to as *ae V*, *ae VII*, and *ae du*. The normal maize starch will be referred to as common corn starch (CCS). All reagents were ACS reagent-grade or better (Fisher Scientific, Fair Lawn, NJ). Granular starch refers to the commercial starches as received, including native lipid. Nongranular starch free of native lipid was prepared by dispersion of granular starch in 90% dimethyl sulfoxide (DMSO) followed by ethanol precipitation and drying at 55°C in a forced-air oven (Klucinec and Thompson 1998). All starch fractions (AM, AP, IM) were prepared from nongranular starch as described by Klucinec and Thompson (1998), using a modification of a two-stage aliphatic alcohol precipitation procedure described by Takeda et al (1986).

Thermal Analysis of Granular and Nongranular Starches and Starch Fractions

Approximately 12 mg (± 0.001 mg) of granular starch, nongranular starch, or starch fraction (dwb) was weighed (AD-4 Autobalance, Perkin-Elmer Corp., Norwalk, CT) in a stainless-steel differential scanning calorimeter pan (Perkin-Elmer), brought to 30% solids (w/w) by adding deionized water, sealed, and stored at room temperature for 24 hr. Samples were heated in a differential scan-

TABLE I
Turbidity ($A_{650\text{nm}}$) of 0.5% Dispersions of Nongranular Starches and Starch Fractions in 20% (v/v) DMSO^a

Starch Sample ^b	Immediately After Sonication	After 5-min Storage in Ice Water	
		Immediately After Storage	After 15 min at Room Temp.
CCS			
Nongranular starch	0.09 (0.04, 0.14)	0.39 (0.27, 0.51)	0.19 (0.07, 0.30)
AP	0.06 (0.07, 0.05)	0.19 (0.24, 0.14)	0.08 (0.10, 0.06)
IM	0.04 (0.03, 0.05)	0.13 (0.15, 0.10)	0.07 (0.06, 0.07)
AM	0.12 (0.01, 0.23)	1.78 (1.62, 1.94)	nd ^c
<i>ae du</i>			
Nongranular starch	0.11 (0.12, 0.11)	0.72 (0.89, 0.55)	0.16 (0.17, 0.15)
AP	0.11 (0.16, 0.05)	0.18 (0.24, 0.11)	0.10 (0.13, 0.06)
IM	0.01 (0.00, 0.03)	0.40 (0.50, 0.30)	0.10 (0.12, 0.09)
AM	0.12 (0.05, 0.19)	2.50 (2.49, 2.51)	nd
<i>ae V</i>			
Nongranular starch	0.08 (0.05, 0.11)	1.34 (0.97, 1.72)	0.21 (0.13, 0.29)
AP	0.08 (0.06, 0.09)	0.44 (0.56, 0.31)	0.17 (0.18, 0.15)
IM	0.06 (0.05, 0.08)	0.51 (0.55, 0.47)	0.15 (0.10, 0.19)
AM	0.12 (0.04, 0.20)	1.82 (1.38, 2.26)	nd
<i>ae VII</i>			
Nongranular starch	0.07 (0.06, 0.09)	1.86 (1.65, 2.07)	nd
AP	0.07 (0.07, 0.07)	0.28 (0.38, 0.18)	0.13 (0.14, 0.12)
IM	0.08 (0.04, 0.13)	0.84 (0.60, 1.08)	0.46 (0.22, 0.70)
AM	0.12 (0.04, 0.19)	2.15 (1.75, 2.54)	nd

^a DMSO = dimethyl sulfoxide. Values are the average of two determinations (in parentheses immediately below the average).

^b Common corn starch. AP = amylopectin; IM = intermediate material; AM = amylose.

^c Not determined. A clear upper layer and a turbid lower layer were observed.

ning calorimeter (DSC-7, Perkin-Elmer) from 5 to 180°C at 10°C/min and quench-cooled ($\approx 150^\circ\text{C}/\text{min}$). An empty stainless-steel pan was used as a reference. Gelatinized granular starches were either reheated immediately from 5 to 180°C at 10°C/min or reheated after storage at 4°C for 3, 7, or 14 days. Each nongranular starch and starch fraction heated to 180°C was either reheated immediately or reheated after storage at 4°C for seven days. Enthalpy of the retrogradation endotherms was calculated (7 Series Software, Perkin-Elmer). Temperature and enthalpy calibrations were made using indium. Duplicate samples were analyzed for each starch and storage-time combination.

Turbidity Measurements of Nongranular Starches and Starch Fractions

The turbidity of each nongranular starch and starch fraction was examined using the method of Adkins and Greenwood (1966) with modifications. The turbidity of a starch dispersion was defined as absorbance at 650 nm. Each nongranular starch and starch fraction (7.5 mg, dwb) was redispersed in 0.3 mL of 90% DMSO in a microcentrifuge tube by heating mixtures for 3 hr in a boiling water bath with intermittent agitation. After dispersion, deionized water (1.2 mL) was added, and the mixture was gently mixed immediately, resulting in a 0.5% starch dispersion.

The absorbance of each 1.5-mL glass cuvette was zeroed with a 20% DMSO solution. Cuvettes and contents were sonicated (model SC-50T; Sonicor Instrument Co., Copaugue, NY) for 5 min to remove air bubbles before absorbance measurements (Gilford Instrument Laboratories, Oberlin, OH) were taken at room temperature. Cuvettes containing dispersions were immersed in an ice water

bath for 5 min, and absorbance was remeasured after quickly drying the outside of the cuvette. The absorbance of dispersions was measured every 5 min for 15 min while standing at room temperature (22°C). Two independently prepared dispersions of each nongranular starch and starch fraction were analyzed.

Starch Gel Preparation and Rheological Examination of Nongranular Starches and Starch Fractions

For each nongranular starch, AP or AM (750 mg, dwb) was added to a glass screw-cap tube. With the tube held at an angle and the starch spread along the length of the tube, 2.0 mL of 100% DMSO (90°C) was added quickly to the tube, and the tube was capped. The contents of the tubes were immediately mixed, and the tubes were placed in a boiling water bath for 1.5 hr, inverted several times, and heated for an additional 1.5 hr. After heating, the redispersed starch samples were brought to a volume of 10 mL with hot (90°C) water and vigorously shaken until the concentrated starch-DMSO mixture and water were thoroughly mixed (<1 min). While hot, the mixture was poured into a gel-casting apparatus used to study dynamic rheological properties (Fig. 1), based on the design of Yuan and Thompson (1998). Dispersed starch was allowed to cool for 5 min at room temperature, at which time the gap in the tubing (Fig. 1) was sealed with melted paraffin wax. The apparatus was stored vertically at 4°C for seven days before rheological analysis.

The dynamic elastic modulus (G') of each gel was measured with a controlled strain rheometer (RFS II, Rheometrics, Piscataway, NJ) in parallel plate (5 cm diameter) geometry, based on the method of Eliasson and Kim (1992) as described by Yuan and Thompson (1998). After seven days, gels were removed from storage at 4°C, Plexiglas plates were separated, and gels were placed on the lower surface of the rheometer. The upper plate was brought into contact with the gel surface until the normal force of the gel counteracted the force of the upper plate on the gel surface. The gel sample was trimmed to the dimension of the top plate with a razor blade, and a thin film of vacuum pump oil (Welch Vacuum Technology, Skokie, IL) was applied to the exposed surface of the gel to minimize water evaporation during the test. The lower plate was heated from 6 to 70°C at 5°C/min with an environmental control circulator (Rheometrics). During the heating process, G' was measured every 20 sec at 5 rad/sec. AP and nongranular starches were tested at 2% strain, and samples of each AM were tested at 0.2% strain. Strains were within the linear viscoelastic region. Two independently prepared gels were examined from each nongranular starch, AP, and AM. For each gel, a ratio of G' at 70°C ($G'_{70^\circ\text{C}}$) to G' at 6°C ($G'_{6^\circ\text{C}}$) was used to calculate the percentage of G' remaining at 70°C.

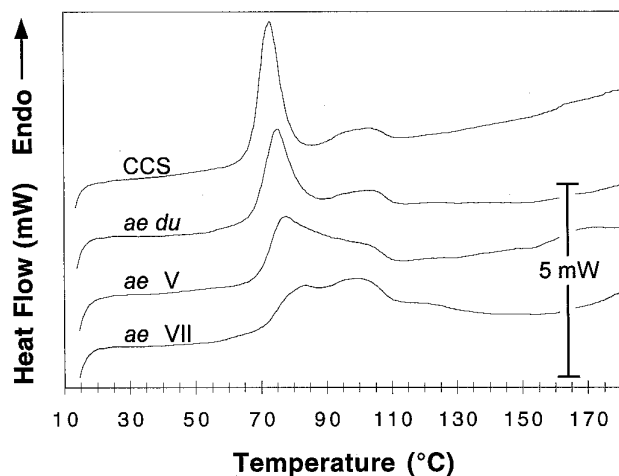


Fig. 2. Differential scanning calorimetry thermograms of gelatinization of common corn (CCS) and high-amylose (*ae du*, *ae V*, *ae VII*) starches (30%, w/w).

RESULTS

Turbidity of Nongranular Starches and Starch Fractions

All dispersions had low turbidity before cooling in an ice-water bath (Table I). After cooling the AM dispersions from each starch in the ice-water bath, samples became highly turbid (Table I). Ap-

TABLE II
Enthalpy^a (ΔH , J/g) of Granular Starches After Initial Heating and Heating After Storage^b

Starch Sample ^c	ΔH on Initial Heating ^d	ΔH on Reheating ^e			
		Immediate Rescan	After 3 Days	After 7 Days	After 14 Days
CCS	19.5 ± 0.14A	2.8 ± 0.24a,A	7.5 ± 0.57b,A	9.1 ± 0.13c,A	10.6 ± 0.20d,A
<i>ae du</i>	24.8 ± 2.01B	4.8 ± 0.71a,A	10.4 ± 1.36b,B	10.6 ± 0.05bc,A	12.7 ± 0.53c,B
<i>ae V</i>	20.6 ± 2.31AB	5.4 ± 1.42a,A	10.2 ± 0.26b,B	13.1 ± 0.44c,B	14.5 ± 0.03c,C
<i>ae VII</i>	22.7 ± 1.03AB	8.4 ± 1.22a,B	9.7 ± 0.91ab,AB	9.7 ± 0.50ab,A	11.0 ± 0.13b,A

^a Enthalpy includes thermal transitions associated with gelatinization and dissociation of amylose-lipid complexes.

^b Values are mean ± SD of two separate analyses. Within each row, values followed by the same lowercase letter are not significantly different at $\alpha = 0.05$ by LSD analysis. Enthalpy of the initial heating was not included in the analysis. Within each column, values followed by the same uppercase letter are not significantly different at $\alpha = 0.05$ by LSD analysis.

^c Common corn starch.

^d Fig. 2.

^e Fig. 3.

proximately 5 min after the cuvette containing each AM was removed from the ice-water bath and held at room temperature, a clear upper layer in the cuvette was apparent above a turbid region. For these samples, turbidity could not be determined by our method (Table I). After cooling the AP and IM from each starch in the ice-water bath, samples became slightly turbid (Table I). The turbidity of cooled AP and IM from each starch was lost after samples were placed in air at room temperature and held for 15 min (Table I). Nongranular starch from CCS, *ae du*, and *ae V* developed higher turbidity than AP and IM from the respective samples when nongranular starches were held in an ice-water bath, but the turbidity of the nongranular starches also was lost when subsequently held at room temperature (Table I). Nongranular *ae VII* developed the highest turbidity of all nongranular starches after being held in an

ice-water bath, and a clear upper layer became apparent after 15 min in air at room temperature (Table I).

Thermal Analysis of Granular and Nongranular Starches and Starch Fractions

Native granular starches. Two endotherms were observed during initial heating of CCS (Fig. 2). The lower temperature endotherm was attributed to loss of native starch double helices (Cooke and Gidley 1992), and the higher temperature endotherm was attributed to melting of amylose-lipid complexes. A multicomponent endotherm, interpreted as containing both events observed for CCS, was observed for each of the three HAS (Fig. 2; Table II).

Endotherms were observed during immediate reheating of each granular starch after initial heating (Fig. 3). For CCS and *ae du*, endotherms observed between 80 and 110°C were attributed to melting of amylose-lipid complexes. For *ae V* and *ae VII*, endotherms observed over a broader temperature range, between ≈55 and

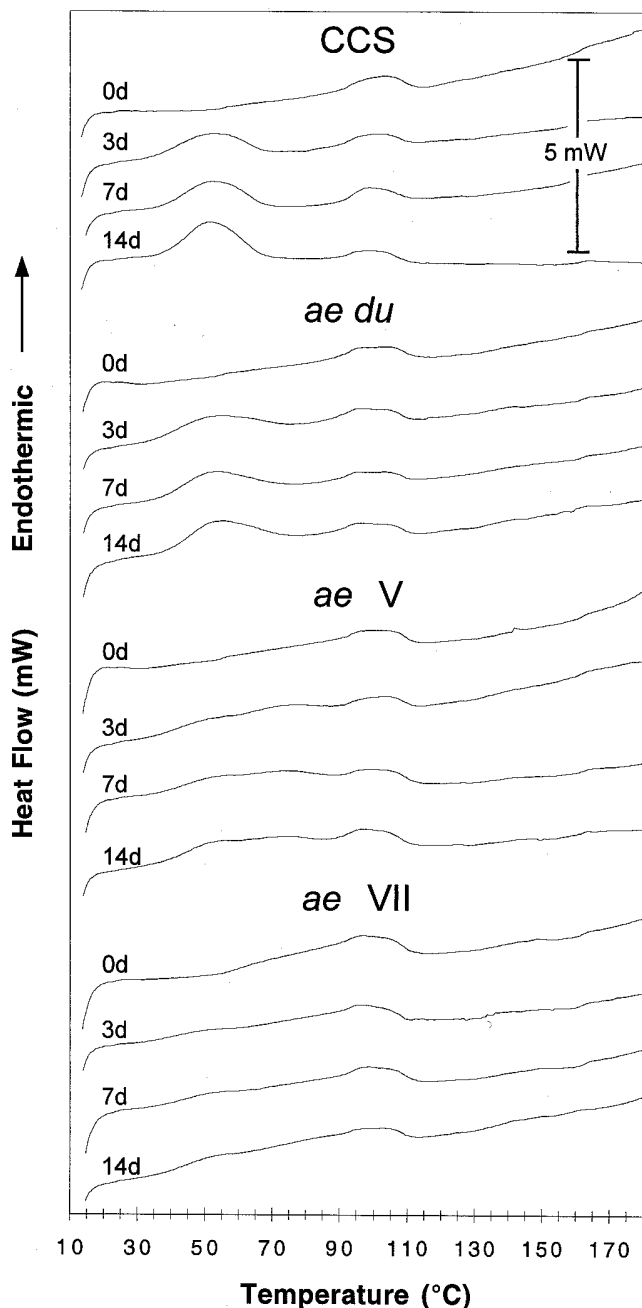


Fig. 3. Differential scanning calorimetry thermograms of common corn (CCS) and high-amylose (*ae du*, *ae V*, *ae VII*) starches (30%, w/w). After initial heating to 180°C, samples were immediately reheated (0 days [d]) or stored for varying lengths of time (3, 7, and 14 days) at 4°C.

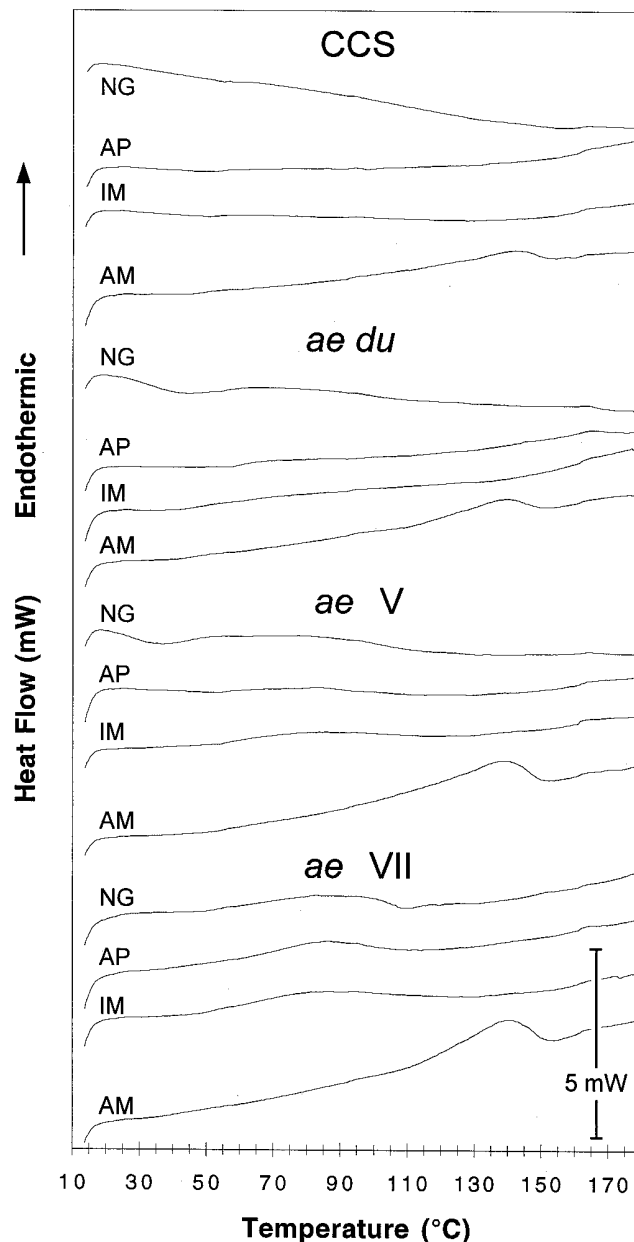


Fig. 4. Differential scanning calorimetry thermograms of immediately reheated nongranular (NG) starches (common corn [CCS], *ae du*, *ae V*, *ae VII*) and their respective starch fractions (AP, IM, AM).

110°C, were attributed to a combination of loss of rapidly retrograded starch double helices and melting of amylose-lipid complexes. During storage at 4°C after initial heating, CCS developed an endotherm between 30 and 70°C (Fig. 3; Table II). Under the same conditions, *ae du* starch developed an endotherm beginning at 30°C that extended to higher temperatures and partially overlapped with the endotherm attributed to melting of amylose-lipid complexes (Fig. 3; Table II); *ae V* and *ae VII* starches also developed endotherms beginning at 30°C. The *ae V* and *ae VII* starch endotherms extended to higher temperatures than *ae du* starch endotherms and, as a result, overlapped more extensively with the amylose-lipid complex endotherm (Fig. 3; Table II).

Nongranular starches and starch fractions. During immediate reheating of AM from each starch, an endotherm with a peak temperature near 140°C was observed (Fig. 4; Table III). During immediate reheating of AP from CCS and *ae du*, no endotherm was observed (Fig. 4). However, for immediately reheated AP from

ae V and *ae VII*, an endotherm was observed between 50 and 120°C (Fig. 4; Table III). During immediate reheating of nongranular CCS, no endotherm was observed (Fig. 4); however, for immediately reheated nongranular *ae du*, *ae V*, and *ae VII*, an endotherm was observed between 50 and 120°C (Fig. 4; Table III). For nongranular *ae du* and *ae V*, the enthalpy observed after immediate reheating was greater than was observed for AP and IM from the respective starches (Table III). No endotherms with a peak at ≈140°C were observed for any nongranular starch during immediate reheating (Fig. 4).

After seven days at 4°C, endotherms for AM were similar in temperature range and enthalpy to those observed during immediate reheating (Fig. 5; Table III). For AP and IM from CCS, endotherms were observed <70°C. For AP and IM from *ae du*, endotherms slightly broader than those of CCS, AP, and IM were observed <90°C (Fig. 5; Table III). Endotherms for AP and IM from *ae V* and *ae VII* were much broader, between 30 and 120°C (Fig. 5; Table III). For all starches, the endotherm for nongranular starch was observed over the same temperature range as the respective AP and IM, and no endotherm with a peak at ≈140°C was observed for any nongranular starch (Fig. 5; Table III).

Dynamic Oscillatory Rheometry of Starch Gels from Nongranular Starch and Starch Fractions

Figure 6 shows the change in G' as temperatures increase from 6 to 70°C for 7.5% starch gels from nongranular starches and the respective AP and AM. The ratio of G' at 70°C ($G'_{70^\circ\text{C}}$) to G' at 6°C ($G'_{6^\circ\text{C}}$) is shown in Table IV. G' for CCS AP fell markedly beginning at ≈40°C and ended near 60°C, reaching an apparent plateau. Although G' for *ae V* AP was much greater at 6°C than for CCS AP, a plateau also was apparent by 60–65°C. G' of *ae du* AP began at a high level, but by 70°C, it dropped to the level of CCS AP. G' for *ae VII* AP began at a high level and decreased gradually as temperatures increased to 70°C. G' of AM gels was

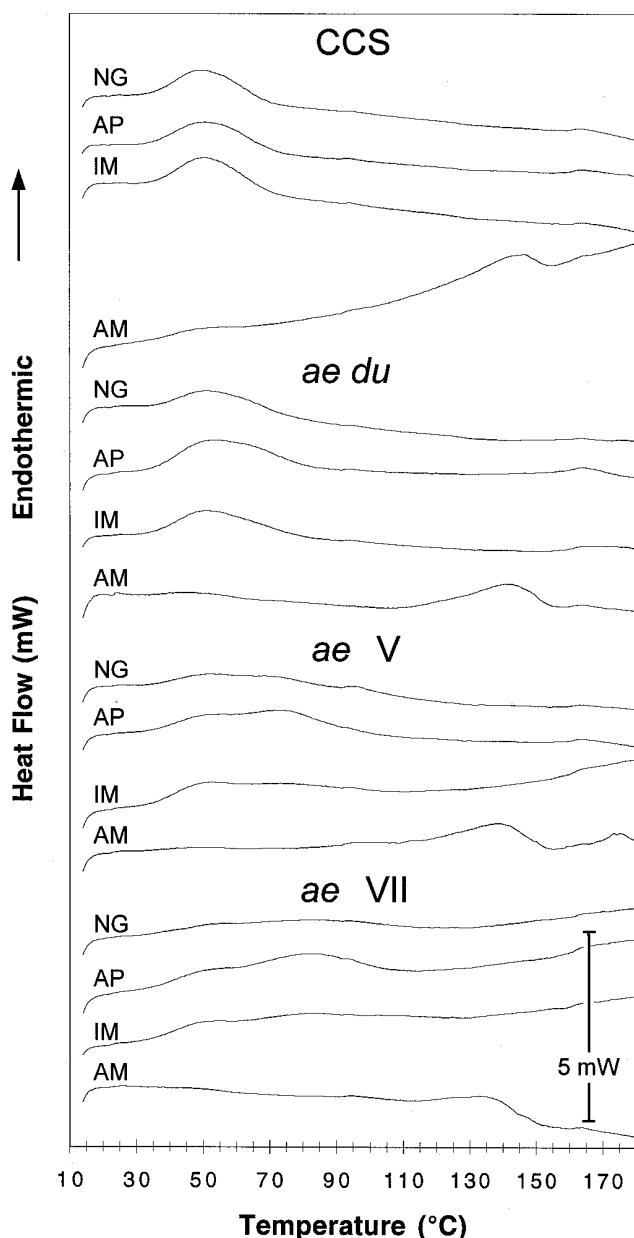


Fig. 5. Differential scanning calorimetry thermograms of nongranular (NG) starches (common corn [CCS], *ae du*, *ae V*, *ae VII*) and their respective starch fractions (AP, IM, AM) after storage for seven days at 4°C.

TABLE III
Retrogradation Enthalpy (ΔH , J/g) of Nongranular Starches and Starch Fractions After Initial Heating to 180°C^a

Starch Sample ^b	ΔH of Immediate Rescan ^c	ΔH After 7 Days Storage at 4°C	
		First Heating to 180°C ^d	Immediate Rescan to 180°C
CCS			
Nongranular starch	nd ^e	10.0 ± 0.23B	nd
AM	nd	11.2 ± 1.00B	nd
IM	nd	11.3 ± 0.21B	nd
AM	5.2 ± 0.39a	7.2 ± 0.41b,A	6.4 ± 0.01b
<i>ae du</i>			
Nongranular starch	6.9 ± 1.26ab,B	10.8 ± 1.64b,A	5.2 ± 1.49a,A
AM	nd	16.5 ± 1.72B	nd
IM	nd	11.5 ± 0.34A	nd
AM	4.5 ± 0.34a,A	8.2 ± 0.80ab,A	13.0 ± 2.90b,B
<i>ae V</i>			
Nongranular starch	8.1 ± 1.45a,C	12.4 ± 0.98b,A	9.6 ± 1.28ab,C
AP	2.3 ± 0.51a,A	18.4 ± 1.30b,B	2.1 ± 1.42a,A
IM	4.5 ± 0.41a,B	12.7 ± 1.4b,A	6.4 ± 0.43a,B
AM	11.7 ± 0.04a,D	9.7 ± 1.54a,A	10.4 ± 0.86a,C
<i>ae VII</i>			
Nongranular starch	8.1 ± 0.03b,A	10.0 ± 0.02c,A	7.6 ± 0.22a,A
AP	8.2 ± 0.28a,A	16.7 ± 1.01b,B	8.9 ± 0.43a,B
IM	8.9 ± 0.99a,AB	13.8 ± 0.50b,AB	8.77 ± 0.27a,AB
AM	10.2 ± 0.21a,B	10.9 ± 2.66a,A	11.4 ± 0.68a,C

^a Within each row, values followed by the same lowercase letter are not significantly different at $\alpha = 0.05$ by LSD analysis. Within each column for each starch, values followed by the same uppercase letter are not significantly different at $\alpha = 0.05$ by LSD analysis.

^b Common corn starch. AM = amylose; IM = intermediate material; AP = amylopectin.

^c Fig. 4.

^d Fig. 5.

^e None detected. Samples were not included in statistical analysis.

far higher than for the respective AP from each nongranular starch, and the proportion of $G'_{6^\circ\text{C}}$ lost when heated to 70°C was much less (Table IV). G' values for nongranular starch from CCS and *ae du* were similar throughout the heating regime. For nongranular starch from *ae V*, G' decreased less extensively. G' for nongranular starch from *ae VII* began at a much higher value than for the other nongranular starches and also decreased proportionately less when heated. The decrease in G' for nongranular starches occurred over a temperature range similar to the decrease in G' of the respective AP.

DISCUSSION

Turbidity of Nongranular Starches and Starch Fractions

Because complete dispersion of amylose or high-amylose starches in water requires heating to temperatures in excess of 150°C , some investigators have examined the rheological behavior of amylose (Colonna and Morris 1987) or turbidity development of amylose (Takeyama et al 1994) and high-amylose starches (Adkins and Greenwood 1966) in a binary solvent of DMSO and water. In our study, we used a binary DMSO-water solvent to examine both the turbidity development and rheological behavior of nongranular starches and starch fractions.

All of the 0.5% dispersions of nongranular starches and the respective fractions in 20% DMSO had turbidity values <0.2 before they were placed in an ice-water bath (Table I). Because turbidity is related to formation of aggregates with a size similar to the wavelength of light (Gidley and Bulpin 1989), this observation indicates that relatively few large aggregates were present. Adkins and Greenwood (1966) also observed limited turbidity development at 20°C over 40 hr for 0.5% dispersions of waxy and regular maize starches in 25% DMSO. Adkins and Greenwood (1966) noted that waxy and regular maize starch dispersions developed turbidity at 6°C , and turbidity disappeared when samples were warmed afterward to 20°C . In the current study, AM from each starch became highly turbid while in an ice-water bath (Table I), and turbidity was not lost during rewarming at room temperature (Table I), indicating that stable large aggregates had formed. The slight turbidity of AP and IM from each starch was lost during subsequent warming (Table I). Consequently, large aggregates from AP and IM were more thermally labile than aggregates from AM.

Each nongranular starch dispersion became more turbid at 6°C than the respective AP or IM (Table I). This higher level of turbidity may be related to the presence of amylose in each nongranular starch. However, the turbidity that developed in nongranular

CCS, *ae du*, and *ae V* was lost when samples were warmed after removal from the ice-water bath. We suggest that the branched molecules in these nongranular starches prevented formation of the stable aggregates observed for the respective AM. Adkins and Greenwood (1966) observed nearly immediate flocculation of 0.5% dispersions of high-amylose starches in 25% DMSO at 6°C . In our study, this behavior was observed only for nongranular *ae VII*. The difference in the behavior of *ae VII* compared with *ae du* and *ae V* could be attributable to the AM content or AP structure of the starch. The high-amylose starches examined by Adkins and Greenwood (1966) contained between 47 and 63% amylose, determined by precipitation with 1-butanol, similar to the AM content of *ae VII* starch (Klucinec and Thompson 1998), determined using similar methods (49%), and higher than levels observed for *ae du* and *ae V* starches (41 and 38%, respectively [Klucinec and Thompson 1998]). AP structure varied as well among the HAS we studied (Klucinec and Thompson 1998).

Thermal Analysis of Nongranular Starches and Starch Fractions

Cooke and Gidley (1992) suggested that the dissociation of starch double helices into single chains is responsible for the endotherms produced by DSC. For an endotherm to be produced by DSC, a

TABLE IV
Elastic Modulus (G' , Pa) of 7.5% Nongranular Starch and Starch Fraction Gels Containing 20% (v/v) DMSO^a and Relative Residual Order of Gels at 70°C ^b

Starch Sample ^c	$G'_{6^\circ\text{C}}$	$G'_{70^\circ\text{C}}$	% G' Remaining at 70°C
CCS			
Nongranular starch			
Trial 1	9.81×10^2	2.09×10^1	2.1
Trial 2	5.30×10^2	1.29×10^1	2.4
AP			
Trial 1	1.84×10^2	1.23×10^1	6.7
Trial 2	1.65×10^2	1.33×10^1	8.1
AM			
Trial 1	2.11×10^4	1.16×10^4	55
Trial 2	2.22×10^4	1.46×10^4	66
<i>ae du</i>			
Nongranular starch			
Trial 1	0.643×10^3	0.632×10^1	1.0
Trial 2	1.18×10^3	1.91×10^1	1.6
AP			
Trial 1	1.85×10^3	9.24×10^0	0.50
Trial 2	1.51×10^3	8.22×10^0	0.54
AM			
Trial 1	2.56×10^4	1.18×10^4	46
Trial 2	1.87×10^4	0.808×10^4	43
<i>ae V</i>			
Nongranular starch			
Trial 1	0.923×10^3	3.94×10^1	4.3
Trial 2	1.02×10^3	6.80×10^1	6.7
AP			
Trial 1	1.27×10^3	0.967×10^2	7.6
Trial 2	1.46×10^3	1.12×10^2	7.7
AM			
Trial 1	2.64×10^4	1.34×10^4	51
Trial 2	3.57×10^4	1.66×10^4	47
<i>ae VII</i>			
Nongranular starch			
Trial 1	3.85×10^3	0.605×10^3	16
Trial 2	5.72×10^3	1.17×10^3	21
AP			
Trial 1	1.66×10^3	1.02×10^2	6.1
Trial 2	1.95×10^3	1.44×10^2	7.4
AM			
Trial 1	2.75×10^4	1.23×10^4	45
Trial 2	3.66×10^4	1.80×10^4	49

^a G' values are reported for two trials. DMSO = dimethyl sulfoxide.

^b Percent was calculated as $(G'_{70^\circ\text{C}}/G'_{6^\circ\text{C}}) \times 100$.

^c Common corn starch. AP = amylopectin; AM = amylose.

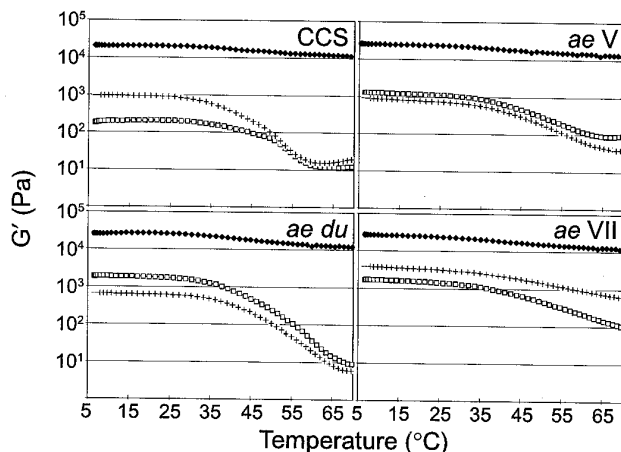


Fig. 6. Gel storage modulus (G') as a function of temperature during heating of gels from nongranular (NG, +) starches (common corn [CCS], *ae du*, *ae V*, *ae VII*) and their respective starch fractions (AP, □; AM, ◆) from 6 to 70°C .

sufficient quantity of double-helical order would be required to dissociate within a sufficiently narrow temperature range. Because the temperature range for the endotherm of double-helix dissociation should be related to helix length, a narrow endotherm would suggest a narrow range of double-helical lengths. An endotherm with a peak at $\approx 140^\circ\text{C}$ was observed during immediate reheating of each AM fraction, indicating the fractions are capable of rapidly reorganizing into a relatively narrow range of long double helices during cooling. Endotherms were not observed during immediate reheating of AP and IM from CCS and *ae du*, indicating the molecules either do not reform double helices or do not reorganize into double helices of a sufficiently narrow range of lengths during cooling.

The inability of branched fractions from CCS and *ae du* to quickly reorganize into double helices may be due to the proportion of short chains (DP <30) in the branched molecules (Klucinec and Thompson 1998), which generally are similar to the proportion of short chains in *wx* starch (Inouchi et al 1987, Yuan et al 1993, Shi and Seib 1995). The endotherms observed between 50 and 120°C during immediate reheating of AP and IM from *ae V* and *ae VII* may be due to the higher proportions of longer chains (DP >30) in these fractions (Klucinec and Thompson 1998). A higher proportion of longer chains also has been observed for *ae wx* starch (Yuan et al 1993, Shi and Seib 1995), which undergoes retrogradation so quickly that enthalpy may be observed by DSC during immediate reheating (Thompson and Blanshard 1995; Fisher and Thompson 1997; Liu and Thompson, *in press*).

After initially heating nongranular or granular starches to 180°C , no endotherm was observed $>120^\circ\text{C}$ during immediate reheating (Figs. 3 and 4), even though 25–50% AM could be recovered from the starches by precipitation (Klucinec and Thompson 1998). However, each fractionated AM readily reorganized into long double helices even after heating to 180°C and rapid ($\approx 150^\circ\text{C}/\text{min}$) cooling. Boltz and Thompson (1999) also observed no endotherm with enthalpy $>120^\circ\text{C}$ after reheating several HAS initially heated to 160 or 180°C ; however, Boltz and Thompson (1999) did observe an endotherm with enthalpy $>120^\circ\text{C}$ for starches initially heated to 120 or 140°C . For nongranular *ae du* and *ae V*, the endotherm observed for immediately reheated nongranular starch was larger than could be accounted for solely by the respective AP and IM (Table III). Gudmundsson and Eliasson (1990) stated that interactions between amylose and amylopectin (as observed by DSC) only occur when amylose exceeds 50% (w/w) of the total starch. Their graphic analysis (Gudmundsson and Eliasson 1990) emphasized statistical interactions (deviation from anticipated linearity), but they did not address the possibility of physical interactions under conditions in which statistical interaction was not observed. Russell (1987) suggested that the reduction in the staling endotherm (generally attrib-

uted to amylopectin) when glycerol monostearate is present might be due to amylose-lipid complex formation, such that any cocrystallization of amylopectin and amylose would be precluded. Fisher and Thompson (1997) and Liu and Thompson (1998, *in press*) demonstrated that retrogradation of waxy-type starches (observed by DSC) is fastest after starch is heated initially to a temperature just above the completion temperature for the DSC endotherm, and retrogradation rate decreases as initial heating temperature increases. The slower retrogradation after higher initial heating temperatures was attributed to the increased disordering of amylopectin chains (Fisher and Thompson 1997; Liu and Thompson 1998, *in press*). When AM is present within the range described in our report, we suggest that after heating to 160 or 180°C or employing solvents sufficient to disorder both AM (Boltz and Thompson 1999) and AP, entanglements may result that prevent the self-association of AM, resulting in mixed double helices between AM and AP during cooling and storage.

The absence of endotherms $>120^\circ\text{C}$ in each of the nongranular starches after seven days of storage at 4°C suggests that development of the high-temperature endotherm attributed to AM was not simply slowed. Endotherms $>120^\circ\text{C}$ also were not observed for gelatinized granular starches stored for 3, 7, or 14 days at 4°C . These observations indicate that the different behavior of AM in nongranular starches compared with each fractionated AM was not due to the absence of granular order in nongranular starches. Boltz and Thompson (1999) also observed no endotherms at $>120^\circ\text{C}$ for similarly heated nongranular and granular starches.

Dynamic Oscillatory Rheometry of Nongranular Starches and Starch Fractions

AP and AM gels. The decrease in G' of each of the AP and AM gels began at $\approx 35^\circ\text{C}$. Gels with a higher $G'_{70^\circ\text{C}}$ (Fig. 6; Table IV) also had greater proportions of DSC enthalpy $>70^\circ\text{C}$ (Fig. 5). Our observations are consistent with the idea that the origin of structure in gels is based on double-helical order (Ring et al 1987, Gidley 1989, Cameron et al 1994). In the current study, no simple relationship was observed between the total enthalpy of each retrograded AP and AM (Table III) and the G' of gels prepared from the fractions (Table IV). Ring et al (1987), Biliaderis and Zawistowski (1990), and Yuan and Thompson (1998) also observed that retrogradation enthalpy was not necessarily an indication of the strength or elasticity of gels.

Although the proportion of starch in double-helical form is not necessarily related to gel structure, junction zones based on double-helical structure are commonly understood to be the basis of starch gel structure. The double helices of a starch gel could be conceptualized as two types: double helices involved in junction zones in a gel and double helices not involved in junction zones. There are

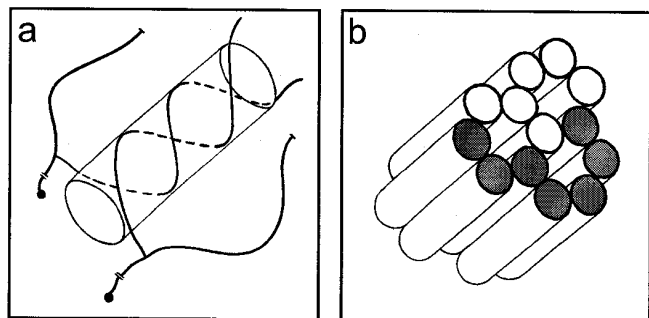


Fig. 7. Possible types of junction zones in starch gels: **A**, intermolecular double helix (type 1 junction zone); **B**, intermolecular aggregate of intramolecular double helices (type 2 junction zone). Black cylinder ends represent double helices from one molecule, and white cylinder ends represent double helices from a different molecule.

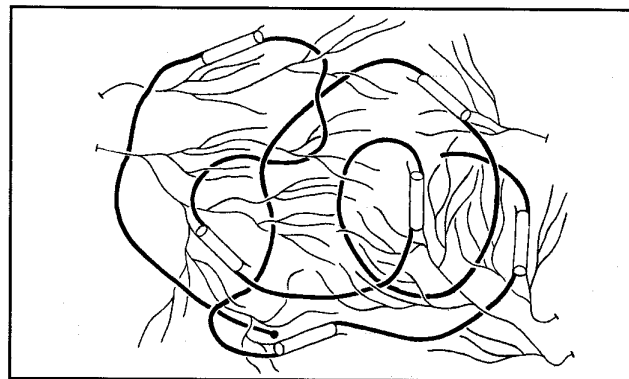


Fig. 8. Possible interactions between short-branched chains of amylopectin and long chains of amylose. Only terminal portions of branched molecules are shown.

two ways in which double-helical structure could form the basis of a junction zone: 1) an individual double helix might itself be a junction zone if the component chains are from two different molecules (forming an intermolecular double helix, as suggested by Cameron et al [1994]) (referred to here as type 1 junction zones); or 2) an intramolecular double helix could participate in a semicrystalline aggregate with double helices from a separate molecule, forming an intermolecular aggregate of double helices serving as a node in an elastic network (Wu and Eads 1993) (referred to here as type 2 junction zones). The distinction is illustrated in Fig. 7.

The stereochemistry of the $\alpha(1\rightarrow6)$ branch point is compatible with a stable double helix formed from two chains connected through the branch point (Imberty and Pérez 1989). Moreover, the branch point may serve as a mechanism to keep two chains in proximity and bring them into proper register. Such a double helix cannot be a type 1 junction zone. It might contribute to a type 2 junction zone or not contribute to the skeletal network of the gel (Ohtsuka et al 1994). Formation of a double helix between two chains not linked by an $\alpha(1\rightarrow6)$ branch point would be at a kinetic disadvantage due to the additional requirement that the two chains must first come into proximity before alignment and stable double helix formation could result. The possibility of chains participating in this sort of double helix would be limited to chains that have the potential to be in proximity to other chains from other molecules. Furthermore, it is reasonable to expect that longer linear regions are more likely to participate in intermolecular double helices based on an increased likelihood for longer chains to form double helices of sufficient length to achieve stability.

The probability that a double helix will aggregate with double helices from a different molecule to form a type 2 junction zone depends on whether the double helix is near the periphery of the molecule, as well as on whether the attachment of the double helix to the rest of the molecule is sufficiently flexible. Longer core chains would produce greater flexibility and encourage greater intermolecular interaction among double helices. Yuan et al (1993) have shown that *ae wx* starch has more long B chains and longer core regions in the chains. Yun and Matheson (1993) showed that both *ae wx* starch and amylopectin of *ae* starch have longer core chains than does amylopectin of normal starch.

The probability that a single chain or double helix in a highly branched molecule is near the periphery of the molecule is related to the size of the molecule. Klucinec and Thompson (1998) showed that the AP of *ae du*, *ae V*, and *ae VII* starch contain molecules of far smaller size than the AP of normal starch. Consequently, the prospect of individual chains (Fig. 7A) or double helices (Fig. 7B) of AP contributing to junction zones is enhanced for HAS.

Nongranular starches. Nongranular starches may be considered mixtures of molecules, most of which can be fractionated as either AM or AP. After dispersing starch in DMSO or heating it to 180°C, it appears that AM and AP interact when diluted or cooled. We suggest that the behavior of nongranular CCS, *ae du*, and *ae V* is the result, in part, of AP-AM interactions. Figure 8 shows the interaction of an AM molecule with portions of more than one AP molecule. The length of the AP-AM double helices that may form is limited by the length of the AP exterior chains. Nevertheless, the probability is high that individual double helices from the interactions serve as type 1 junction zones. These intermolecular double helices also could contribute to type 2 junction zones. Fewer AM-AM interactions would limit the length of double helices, and thus, the nongranular gel would be more like AP than AM in its thermal behavior.

Based on DSC and pulsed low-resolution NMR examination of mixtures of potato maltodextrin and short-chain amylose with an average DP of 32, Schierbaum et al (1992) suggested that amylose interacted with the outer linear chains of branched molecules. Although Leloup et al (1991) considered gels of potato amylose and waxy maize starch molecules (each without physical modifications) as phase-separated systems, they suggested that the differ-

ent molecules may cocrystallize when they come in contact with each other at interfaces. Because DSC endotherms at 140°C were not observed for any nongranular starch in our study, we conclude that AM is not a separate phase, and the normal length of the AM-AM double helices is precluded. Although we cannot rule out the possibility that a wide range of lengths in the AM-AM double helices accounts for the lack of a DSC endotherm at $\approx 140^\circ\text{C}$, we believe it is more likely that AM-AP double helices preclude the formation of long AM-AM double helices, resulting in AM-AP double helices that are lost over the same temperature range as AP-AP double helices.

In the current study, two solvent systems were used: a system containing 20% DMSO in water and a system containing only water. Also, different concentrations of starch were examined in the three methods of analysis (turbidity, DSC, and rheology). Because of the different conditions for each method of analysis, caution must be used when comparing turbidity, thermal behavior, and rheological behavior. Formation of interactions between AM and branched molecules may depend on the specific processing conditions under which both the branched molecules and AM retain no residual order (Boltz and Thompson 1999). Either heating the starches to $\geq 160^\circ\text{C}$ or dispersing starches in 90% DMSO would result in the dissociation of double helices of amylose and branched molecules. With amylose and branched molecules in a highly disordered and dispersed state, cooling of starch or reducing DMSO concentration could promote formation of interactions between branched molecules and amylose, resulting in a gel in which amylose and branched molecules interact before they can phase separate.

Nongranular *ae VII* differed from the other two HAS in that turbidity was not lost when warmed and the G' was the highest at 6°C and retained the highest proportion of G' at 70°C. Although we did not collect rheological data at $>70^\circ\text{C}$, we suspect that G' would continue to decrease until the completion temperature of the nongranular *ae VII* DSC endotherm was reached and, thus, would be largely thermoreversible at $<100^\circ\text{C}$. We suggest that the nature of the AM-AP interaction could be influenced by 1) the proportion of AM to AP; 2) the chain lengths of AP; and 3) the size distribution of AP. Each of the factors may contribute to the more stable gels of nongranular *ae VII*. We intend to explore the importance of these three contributions in future research.

While our manuscript was in preparation, a series of articles was published describing a new and especially high-amylose maize starch, referred to as "low-amylopectin starch" (Case et al 1998, Shi et al 1998, Sidebottom et al 1998). It will be interesting to learn whether AM-AP is high enough in low-amylopectin starch to overcome the effect of AP on AM behavior.

CONCLUSIONS

In mixtures of amylose and branched molecules from common corn starch and high-amylose maize starches, the branched molecules in each starch appear to inhibit the ability of amylose to form long double helices, limiting development of stable aggregates. In such mixtures, the system appears to conform to the thermal behavior and aggregation abilities of the branched molecules, as observed by DSC and dynamic oscillatory rheometry. The retrogradation behavior of each HAS may be due to differences in the proportion of AM-AP, but it appears that other factors, perhaps chain length distribution and molecular size of branched molecules, are important as well. An understanding of the nature of branched molecules, even in high-amylose starches, may be critical to understanding the behavior of unfractionated starches.

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