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Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley

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Abstract

Wheat, triticale, and barley starches have large, disc-shaped A-granules and small, spherical B-granules. In this study, A- and B-granules of these three starches were separated, and their structures and properties were analyzed. Absolute amylose contents of wheat, triticale, and barley A-granules were 30.9%, 28.2%, and 28.1%, respectively, and that of the B-granules were 25.5%, 19.7%, and 23.0%, respectively. High-performance anion-exchange chromatography, equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD), of debranched amylopectins showed that the A-granules had more B_2 chains and lesser short chains (A and B_1 chains) than did the B-granule counterparts. Structure models of the amylopectins of the A- and B-granule starches were proposed, and the relationships between the structures of amylopectin and the shapes of starch granules were discussed. The B-granules displayed higher peak gelatinization temperatures and broader gelatinization temperature ranges than did the A-granules. The retrogradation rates of the B-granules after being stored at 4 °C for 7 days were less than that of the A-granules. The B-granules had higher pasting temperatures, less peak, breakdown, and setback viscosity than did the A-granule counterparts. The pasting properties of reconstitute wheat starch consisting of different proportions of the A- and the B-granules showed that the chemical composition and structure of the starch, instead of the size of the starch granules, controlled the pasting properties of the starch. The differences in the structure of the A- and B-granules suggested that biosynthesis of the two granules differed.

Keywords: A-granule starch; B-granule starch; Starch structure; Starch property; Amylopectin; Barley; Wheat; Triticale

1. Introduction

Wheat, triticale (wheat-rye hybrid), and barley mature endosperms consists of two distinct starch granules, large, disk-shaped A-granules and small, spherical B-granules. The granules of different sizes and shapes are developed in the endosperm during different periods of grain development. The A-granules appear 4 days after anthesis and continue to increase in size throughout the grain-filling period, whereas the B-granules are initiated 12–14 days after anthesis and remain considerably smaller (Bechtel, Zayas, Kaleikau, & Pomeranz, 1990; Karlsson, Olered, & Eliasson, 1983; Morrison & Gadan, 1987). Recent studies using transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) showed that B-granules are present in the protrusions emanating from the A-granule-containing amyloplasts (Langeveld, van Wijk, Stuurman, Kijne, & de Pater, 2000). The amyloplasts are interconnected by these protrusions. The protrusions varied in length from 2 to 30 μm and ranged in width from 0.5 to 1.5 μm, depending on the presence of B-granules, when viewed with a CLSM. These protrusions suggest the presence of a communication system facilitating the coordination of plastid activities. Bechtel et al. (1990) and Raeker et al. (1998) reported that wheat starch showed a trimodal rather than a bimodal distribution, and the third distinct class of small C-granules were initiated 21 days after anthesis. The proportions of small and large starch granules, by weight and by number, differed among genotypes (Li, Vasanthan, Rossnagel, & Hoover, 2001;

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Stoddard, 1999). The number of the A-granules is always smaller than that of the B-granules, but the A-granules represent the major mass of the starch.

The two types of starch granules differ in their structures. The A- and B-granules have different ratios of amylose to lipid contents (McDonald, Stark, Morrison, & Ellis, 1991; Morrison & Gadan, 1987). The B-granules have more lipid content than the A-granules (Meredith, Dengate, & Morrison, 1978; Raeker, Gaines, Finney, & Donelson, 1998; Soulaka & Morrison, 1985). For barley (Takeda, Takeda, Mizukami, & Hanashiro, 1999) and normal and partial waxy wheat (Bertolini, Souza, Nelson, & Huber, 2003), the A-granule starch has a larger amylose content than does the B-granule starch. Studies of the pasting properties of wheat starch show that the A-granule starch has greater peak, final, breakdown, and setback viscosity than does the B-granule starch counterpart (Sahlstrom, Baevre, & Brathen, 2003; Shinde, Nelson, & Huber, 2003).

Peng, Gao, Baga, Hucl, and Chibbar (2000) report that two proteins, having molecular weights of 140 and 145-kDa, preferentially associate with the A-granules in developing and mature wheat, which are isozymes of SBEIc, a 152-kDa isoform of wheat starch-branching enzyme. The 152-kDa SBEIc, is a granule-bound form of SBEI in wheat (Baga, Nair, Repellin, Scoles, & Chibbar, 2000). Because the proteins of 140 and 145-kDa are less or not found in small granules, the authors suggest that these proteins play a role in the development of disk-shaped A-granules.

Granule size affects the physicochemical properties of starch. The different sizes and shapes of the A- and B-granules of wheat, triticale, and barley starches are important characteristics, which determine their end uses. For example, wheat A-granule starch has been used in carbonless copy paper and wheat B-granule starch is good for plastic film filler. Starch granules from different botanical origins differ in morphology. It is not known how morphology of starch is controlled during biosynthesis.

In this study, we analyzed the morphology, crystalline structure, amylose content, branch chain-length distribution of amylopectin, and thermal and pasting properties of the A- and B-granules of wheat, triticale, and barley starches. On the basis of these results, we proposed a model of how the structures of amylopectin relate to the shapes of starch granules.

2. Materials and methods

2.1. Materials

Barley, triticale (NT98424), and wheat (Wesley) grains were provided by Dr. R. A. Graybosch, USDA. Protease from *Aspergillus sojae* and amyloglucosidase from *Rhizopus* were purchased from Sigma Chemical Co. (St. Louis, MO). Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Other chemicals (reagent grade) were used without further processing.

2.2. Isolation of starch

Barley starch was isolated using a method of Song and Jane (2000). Wheat and triticale starches were isolated following the method reported by Kasemsuwan, Jane, Schnable, Stinard, and Robertson (1995). Isolated starches were washed with water and ethanol, and recovered by filtration, using Whatman No. 4 filter paper, before drying in a convection oven at 32 °C for 48 h.

2.3. Separation of the A- and B-granule starch

The A- and B-granules were separated by sedimentation using graduated cylinders (2 L) as described by Takeda et al. (1999). The fraction of 2 h precipitate was collected as large granules and the fraction remaining in the supernatant after 20-h sedimentation was collected as small granules. The fractionation processes were repeated five times for the A-granules and three times for the B-granules. The separated A- and B-granule suspensions were centrifuged at 7000 rpm for 20 min and washed with three volumes of ethanol one time. These starches were recovered by filtration (Whatman No. 4 filter paper) and then dried in a convection oven at 32 °C for 48 h. Microscopic images showed no contamination of granules of other sizes in each fraction.

2.4. Fractionation of amylose and amylopectin

Amylopectin of barley, wheat, and triticale starch was fractionated from amylose and purified by using the amylose/1-butanol complex method (Jane & Chen, 1992).

2.5. Apparent and absolute amylose contents

Apparent amylose contents were determined by measuring iodine affinities (IA) of DMSO-defatted starches using a potentiometric autotitritor (702 SM Titrino, Brinkmann Instrument, Westbury, NY) (Yoo & Jane, 2002). Iodine affinity of 20% for amylose was used for calculation (Takeda & Hizukuri, 1987). The iodine affinity of pure amylopectin was determined following the same method. Absolute amylose contents were determined by subtracting the iodine affinities of amylopectins from that of the defatted whole starches following the method of Takeda, Takeda, and Hizukuri (1983).

2.6. Morphology of starch granules

Scanning electron micrographs (SEM) of starch samples were taken using a scanning electron microscope (JEOL JSM-35, Tokyo, Japan) at the Bessey Microscopy Facility, Iowa State University. The starch samples were coated with gold–palladium (60:40), and the SEM images were

taken at 40 kV (Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994).

2.7. Crystalline structure of starch

X-ray diffraction patterns of the starch samples were obtained using a diffractometer (D-500, Siemens, Madison, WI) with copper Kα radiation (Yoo & Jane, 2002).The following equation was used to determine percentage crystallinity: Crystallinity (%) $= A_c/(A_c + A_a)$ 100, where A_c is the crystalline area on the X-ray diffractogram and A_a is the amorphous area (Hayakawa, Tanaka, Nakamura, Endo, & Hoshino, 1997).

2.8. Amylopectin branch chain-length distribution

Amylopectin was debranched using isoamylase (Jane et al., 1999). Branch chain-length distribution of amylopectin was analyzed using a high-performance anion-exchange chromatography equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Dionex, Sunnyvale, CA) following the method reported by Wong and Jane (1997). A PA-100 anion-exchange analytical column and a guard column (Dionex, Sunnyvale, CA, USA) were used for sample separation. The profile of separation gradient composed of eluent A (100 mM sodium hydroxide) and eluent B (100 mM sodium hydroxide and 300 mM sodium nitrate) was: 0–5 min, 99% A and 1% B; 5–30 min, linear gradient to 8% B; 30–150 min, linear gradient to 30% B; 150–200 min, linear gradient to 45% B. Each sample was analyzed in duplicate.

2.9. Thermal properties

Thermal properties of the native and retrograded starch were analyzed using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with an intracooling II system. Aluminum pans (Perkin-Elmer) were used for the analysis. Starch samples (2 mg, dried starch basis, dsb) were precisely weighed in the sample pans, mixed with distilled water (6 mg), and sealed. The heating rate was at 10 °C per min over the temperature range of 25–120 °C. Indium and zinc were used as the reference standards. Enthalpy change (ΔH), gelatinization onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were measured and calculated by using Pyris software

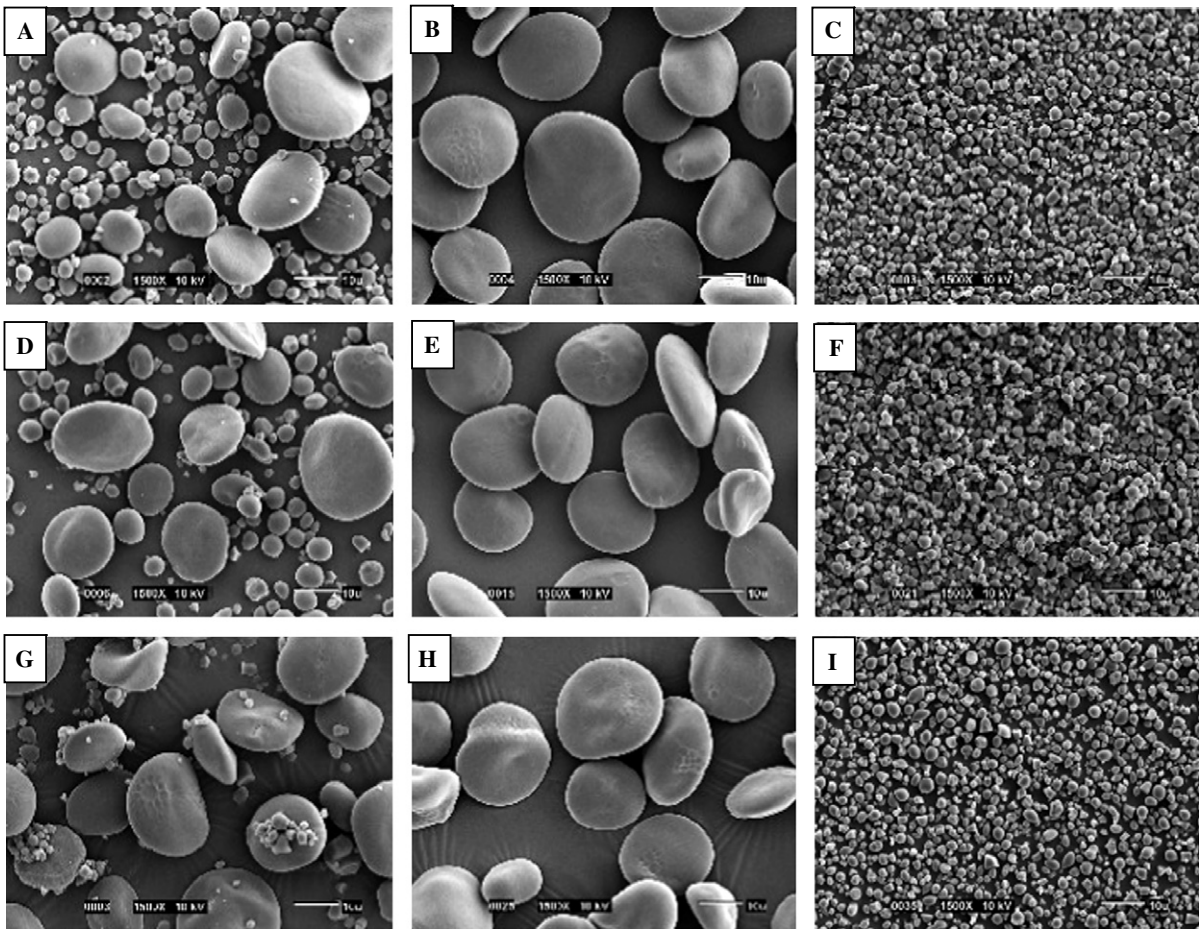

Fig. 1. Scanning electron micrographs of native wheat (A), triticale (D), and barley (G) starch granules, and their fractionated large, A-granules (B, E, and H, respectively) and small, B-granules (C, F, and I, respectively) (Scale bar = 10 μm).

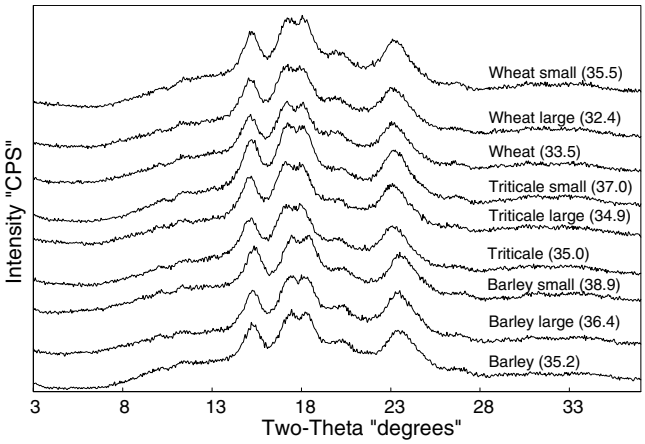

Fig. 2. X-ray diffraction patterns of wheat, triticale, and barley starches, and their fractionated A- and B-granules. Crystallinity (%) was given in parentheses.

Table 1
Amylose contents of wheat, triticale, and barley starches

Sample	Iodine affinity (%)[a]		Amylose content (%)	
	Starch	Amylopectin	Apparent[b]	Absolute[c]
Wheat	6.4 ± 0.1[d]	0.8 ± 0.0	32.0	29.2
Wheat A-granule	6.8 ± 0.1	0.9 ± 0.1	34.0	30.9
Wheat B-granule	5.4 ± 0.0	0.4 ± 0.0	27.0	25.5
Triticale	5.9 ± 0.0	0.7 ± 0.0	29.5	26.9
Triticale A-granule	6.0 ± 0.0	0.5 ± 0.0	30.0	28.2
Triticale B-granule	4.1 ± 0.1	0.2 ± 0.0	20.5	19.7
Barley	6.0 ± 0.1	0.8 ± 0.0	30.0	27.1
Barley A-granule	6.2 ± 0.0	0.8 ± 0.0	31.0	28.1
Barley B-granule	4.9 ± 0.0	0.4 ± 0.0	24.5	23.0

[a] Averaged from at least two analyses.
[b] Apparent amylose content (%) = $IA_{starch}/IA_{amylose} \times 100$. Iodine affinity for pure amylose was 20%.
[c] Absolute amylose content (%) = $(IA_{starch} - IA_{amylopectin})/(IA_{amylose} - IA_{amylopectin}) \times 100$.
[d] ±Standard deviation.

(Perkin-Elmer, Norwalk, CT). The data were averages of a minimum of three replicates of each starch sample. The properties of retrograded starch were analyzed following the same method as measurement of gelatinization using the same gelatinized starch samples that had been stored at 4 °C for 7 days.

2.10. Pasting properties

Pasting properties of the starch were determined by using a Rapid Visco-Analyzer (RVA) (RVA-4, Foss North America, Eden Prairie, Minnesota, USA). Each starch suspension (8%, dsb, w/w; 28 g total weight) was equilibrated at 50 °C for 1 min and then heated at a rate of 6 °C/min to 95 °C and then maintained at that temperature for 5 min. The sample was then cooled to 50 °C at a rate of 6 °C/min. A rotating speed of the paddle (160 rpm) was used except the paddle speed was 960 rpm at the first 10 s.

3. Results and discussion

3.1. Granule morphology and crystalline structure

Wheat, triticale, and barley starch granules showed bimodal size distributions (Fig. 1A, D, and G, respectively). The scanning electron micrographs of the A- and B-granules isolated from these three starches are also shown in Fig. 1. The A-granules of the starches displayed a disk shape with diameters of 10–35 μm (Fig. 1B, E, and H, respectively), and the isolated B-granules displayed a spherical shape with diameters of about 2 μm (Fig. 1C, F, and I, respectively). The morphology of the starch was in agreement with previously reported results (Jane et al., 1994; Song & Jane, 2000; Yoo & Jane, 2002). The scanning electron micrographs showed that wheat starch had a greater proportion of small granules than did triticale and barley starches.

The whole starch and the separated A- and B-granules of wheat, barley, and triticale starches all displayed typical A-type X-ray diffraction patterns (Fig. 2). All the B-granules possessed greater crystallinity (35.5–38.9%) than did the A-granule counterparts (32.4–36.4%, Fig. 2). Tang, Watanabe, and Mitsunaga (2002) reported that the crystallinity of normal barley B-granules (23.9%) was greater than that of the A-granules (20.3%), whereas for waxy barley starch, the waxy barley A-granules had greater crystallinity (36.6%) than did the B-granules (33.0%). The difference in percentage crystallinity between the normal and waxy barley starches is attributed to the amylose content of normal barley starch. Amylose in the starch granules is amorphous; thus, normal starch displays less percentage crystallinity than the waxy starch counterpart. The different crystallinity of normal barley starch obtained in the two laboratories could result from sample preparation and techniques used for measurement and calculation.

3.2. Apparent and absolute amylose content

Both apparent and absolute amylose contents of the A- and B-granule starches of wheat, triticale, and barley were analyzed, and the results are shown in Table 1. The A-granule starches consisted of more amylose (34.0%, 30.0%, and 31.0% apparent amylose for wheat, triticale, and barley starch, respectively) than the B-granule starch (27.0%, 20.5%, and 24.5%, respectively) (Table 1). These results agreed with previously reported data for barley (Takeda et al., 1999) and for normal and partial waxy wheat starch (Bertolini et al., 2003). The values of the apparent amylose contents were larger than that of the absolute amylose content (30.9%, 28.2%, and 28.1% for the A-granules of wheat, triticale, and barley starch, respectively, and 25.5%, 19.7%, and 23.0% for the B-granules, respectively). The difference between the apparent and the absolute amylose contents was attributed to that the long branch-chains of amylopectin bound iodine during the iodine affinity analysis and inflated the value of the

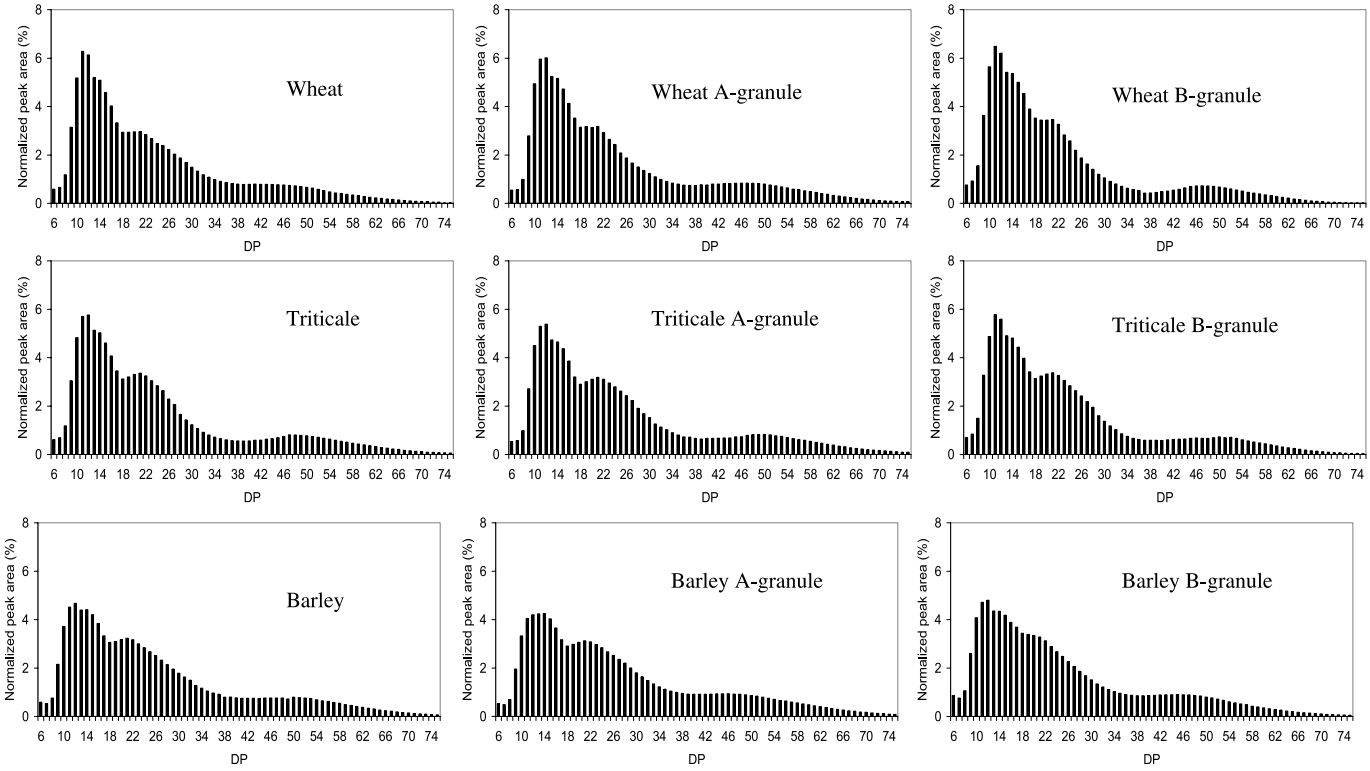

Fig. 3. Amylopectin branch chain-length distributions of the whole starch, the A- and the B-granules of wheat, triticale, and barley starches. The analysis was done by using a high-performance anion-exchange chromatograph system equipped with an on-line enzyme column and a pulsed amperometric detector (HPAEC-ENZ-PAD) (DP = degree of polymerization).

Table 2
Branch chain-length distributions of amylopectins of wheat, triticale, and barley starches[a]

Sample	Peak DP		Average CL	Distribution (%)				
	I	II		DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP ⩾ 37
Wheat	11	43	23.3	5.6 ± 0.1[b]	23.2 ± 0.3	42.0 ± 0.6	18.0 ± 1.3	16.8 ± 1.6
Wheat A-granule	12	47	24.2	4.9 ± 0.0	21.8 ± 0.0	43.3 ± 0.3	15.1 ± 0.2	19.7 ± 0.1
Wheat B-granule	11	47	21.8	6.9 ± 0.1	25.2 ± 0.1	46.8 ± 0.4	13.5 ± 0.2	14.2 ± 0.5
Triticale	12	47	23.8	5.5 ± 0.1	21.8 ± 0.2	44.4 ± 0.1	16.1 ± 0.2	17.7 ± 0.2
Triticale A-granule	12	50	25.0	4.8 ± 0.1	20.0 ± 0.3	41.9 ± 0.5	18.2 ± 0.5	19.9 ± 0.3
Triticale B-granule	11	50	23.4	6.3 ± 0.2	22.6 ± 0.2	43.8 ± 0.7	17.3 ± 0.5	16.4 ± 0.0
Barley	12	50	25.7	4.0 ± 0.0	16.9 ± 0.1	41.8 ± 0.3	21.0 ± 0.2	20.3 ± 0.2
Barley A-granule	12	50	26.7	3.7 ± 0.0	15.3 ± 0.2	40.3 ± 0.2	21.4 ± 0.2	22.9 ± 0.2
Barley B-granule	12	50	24.9	5.3 ± 0.2	18.9 ± 0.2	42.6 ± 0.1	18.4 ± 0.0	20.0 ± 0.1

[a] The results were computed from the histograms shown in Fig. 3.
[b] ±Standard deviation.

amylose content. The amylose content of starch is, in general, proportional to the granule size and maturity of starch (Jane & Shen, 1993; Kulp, 1973; Meredith et al., 1978; Pan & Jane, 2000). Because amylose is amorphous in the starch granule, the larger amylose content of the A-granule starch is likely to result in a lesser percentage of the crystallinity of the A-granule starch than the B-granule starch.

3.3. Branch chain-length distribution of amylopectin

Branch chain-length distributions of amylopectin molecules of various starch samples, obtained by using HPAEC-ENZ-PAD, are shown in Fig. 3, and the computed results are summarized in Table 2. The B-granules had shorter average branch-chain length than did the respective A-granules (Table 2). Branch chain-length distributions of the wheat, triticale, and barley B-granules showed more short chains (A and B_1 chains, DP 6–12, 25.2%, 22.6%, and 18.9%, respectively, and DP 13–24, 46.8%, 43.8%, and 42.6%, respectively), and lesser long B_2 chains (DP > 37, Table 2) than did the A-granules (DP 6–12, 21.8%, 20.0%, and 15.3%, respectively; DP 13–24, 43.3%, 41.9%, and 40.3%, respectively) (Table 2). Details of the chain length differences are shown in the differential histograms of the chain-length distributions between the B- and A-granules

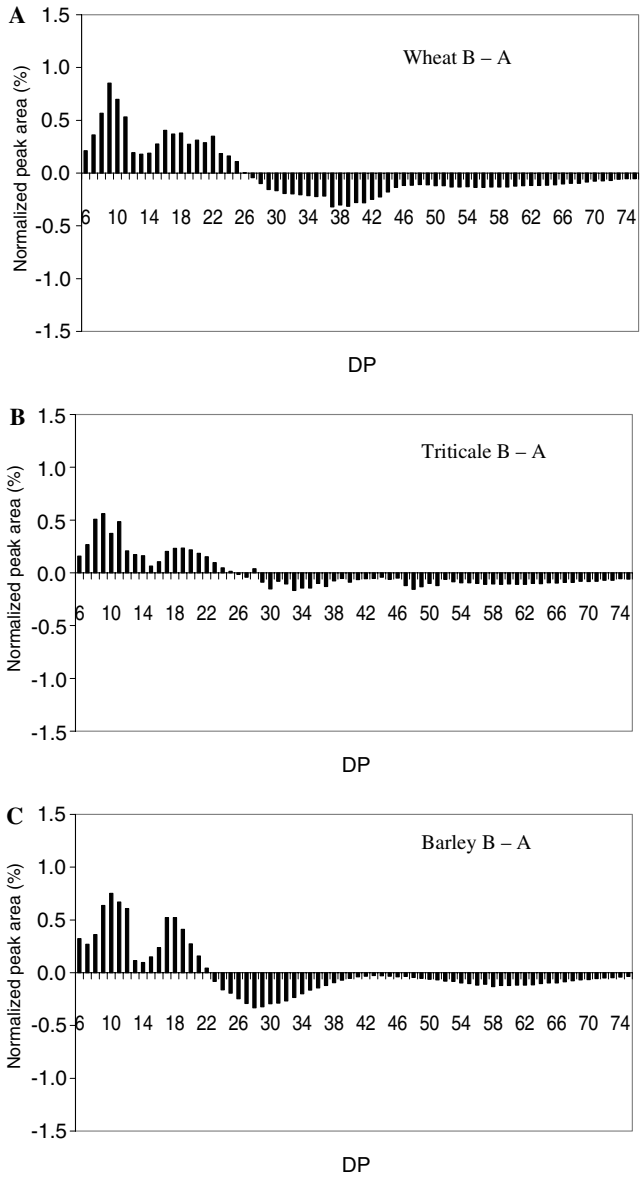

Fig. 4. Differential histograms of amylopectin branch chain-length distributions between the B- and the A-granules; (A) wheat, (B) triticale, and (C) barley (DP = degree of polymerization).

(Fig. 4A–C). The results indicated that the B-granules of wheat and triticale starches consisted of more branch chains of DP 6–25 and lesser branch chains of DP ⩾ 26 than did the A-granules (Fig. 4A and B). Barley B-granules had more branch chains of DP 6–22 and lesser branch chains of DP ⩾ 23 than did the A-granules (Fig. 4C). These results showed that the A-granules consisted of more B_2 chains but lesser A and B_1 chains than the B-granules. The differences between the A- and the B-granules were substantially larger than that between the large and small granules of cornstarch (data not shown). These data suggest that amylopectin molecules of the A- and B-granules have distinct fine structures, which are likely genetically controlled during their biosynthesis (Peng et al., 2000).

3.4. Thermal properties

Gelatinization properties of the starches are shown in Table 3. The B-granules had broader ranges of gelatinization temperatures ($T_c - T_o$) than did the A-granules (Table 3). These findings agreed with the report that the small granule starch had a broader gelatinization temperature range than the unfractionated starch (Eliasson & Karlson, 1983). The gelatinization peak temperatures (T_p) of the B-granules were higher than that of the A-granules (Table 3). No significant difference was found between the gelatinization enthalpy changes of the A- and B-granule starches (Table 3). The DSC thermograms of all starches showed a melting peak of amylose-lipid complex with the onset temperature between 93.2 and 95.5 °C, and conclusion temperature between 102.2 and 109.2 °C. Triticale starch exhibited much smaller enthalpy changes of the amylose-lipid complex melting peak than did wheat and barley starches, indicating less lipid content of triticale starch. Wheat and barley B-granule starch displayed greater enthalpy changes of the amylose-lipid melting peaks than did the A-granule starch counterparts. These results agreed with the reports of Takeda et al. (1999) and Bertolini et al. (2003), reflecting more phospholipids present in the B-granules.

Table 3
Gelatinization properties of wheat, triticale, and barley starches[a]

Sample	Peak I[b]				Peak II[c]			
	T_o	T_p	$T_c - T_o$	ΔH (J/g)	T_o	T_p	T_c	ΔH (J/g)
Wheat	61.7 ± 0.1	65.3 ± 0.1	7.6	12.4 ± 0.2	93.2 ± 0.7	98.6 ± 0.2	102.6 ± 0.3	1.0 ± 0.2
Wheat A-granule	61.2 ± 0.1	64.3 ± 0.3	6.9	11.7 ± 0.3	93.8 ± 1.3	98.4 ± 0.6	102.5 ± 0.7	0.9 ± 0.0
Wheat B-granule	57.9 ± 0.3	64.7 ± 0.6	9.6	12.1 ± 0.1	93.7 ± 0.3	99.6 ± 0.8	105.1 ± 0.5	1.3 ± 0.1
Triticale	60.7 ± 0.1	64.2 ± 0.2	7.7	12.6 ± 0.4	93.4 ± 0.5	98.1 ± 0.4	102.8 ± 1.2	0.6 ± 0.1
Triticale A-granule	60.4 ± 0.1	63.5 ± 0.2	7.1	12.6 ± 0.2	95.5 ± 0.5	98.6 ± 0.2	102.2 ± 0.5	0.3 ± 0.2
Triticale B-granule	57.2 ± 0.3	64.1 ± 0.2	13.4	12.1 ± 0.4	95.0 ± 2.2	99.4 ± 1.4	103.4 ± 1.7	0.2 ± 0.2
Barley	57.9 ± 0.3	62.6 ± 0.1	10.0	12.6 ± 0.3	94.1 ± 0.2	99.5 ± 0.5	103.3 ± 0.3	0.9 ± 0.1
Barley A-granule	57.0 ± 0.1	61.5 ± 0.4	9.4	12.2 ± 0.7	94.1 ± 0.4	99.5 ± 0.4	103.4 ± 0.7	0.9 ± 0.2
Barley B-granule	58.2 ± 0.2	66.3 ± 0.4	15.4	12.7 ± 1.2	94.4 ± 1.2	101.9 ± 1.2	109.2 ± 1.7	2.1 ± 0.2

[a] T_o, T_p and T_c = onset, peak and conclusion temperatures (°C) of endotherm. ΔH = enthalpy change of gelatinization and melting of amylose-lipid complex. Values are mean ± standard deviation.
[b] Gelatinization.
[c] Melting of amylose-lipid complex.

Table 4
Retrogradation properties of wheat, triticale, and barley starches[a]

Sample	T_o	T_p	T_c	ΔH (J/g)	Retrogradation (%)[b]
Wheat	40.6 ± 1.9	51.2 ± 2.2	61.0 ± 0.6	4.3 ± 0.1	34.6
Wheat A-granule	38.8 ± 0.1	49.6 ± 0.4	60.3 ± 0.6	4.2 ± 0.4	35.9
Wheat B-granule	38.7 ± 0.8	49.1 ± 0.7	57.2 ± 0.7	3.0 ± 0.4	24.8
Triticale	43.8 ± 0.2	53.6 ± 0.3	60.1 ± 0.4	4.0 ± 0.1	31.7
Triticale A-granule	43.1 ± 2.0	53.1 ± 1.0	59.9 ± 0.6	3.7 ± 0.3	29.4
Triticale B-granule	41.6 ± 0.5	51.1 ± 1.5	58.2 ± 0.6	2.7 ± 0.2	22.3
Barley	43.1 ± 0.8	52.8 ± 0.5	59.3 ± 0.3	5.5 ± 0.3	43.7
Barley A-granule	42.8 ± 0.6	52.6 ± 0.4	59.7 ± 0.6	5.5 ± 0.8	45.1
Barley B-granule	44.1 ± 0.8	53.4 ± 0.4	59.3 ± 0.6	4.7 ± 0.2	37.1

[a] T_o, T_p, and T_c = onset, peak and conclusion temperatures (°C) of endotherm. ΔH = enthalpy change of dissociation of retrograded starch. Values are mean ± standard deviation.
[b] %Retrogradation = (enthalpy change of retrograded starch/enthalpy change of native starch) × 100.

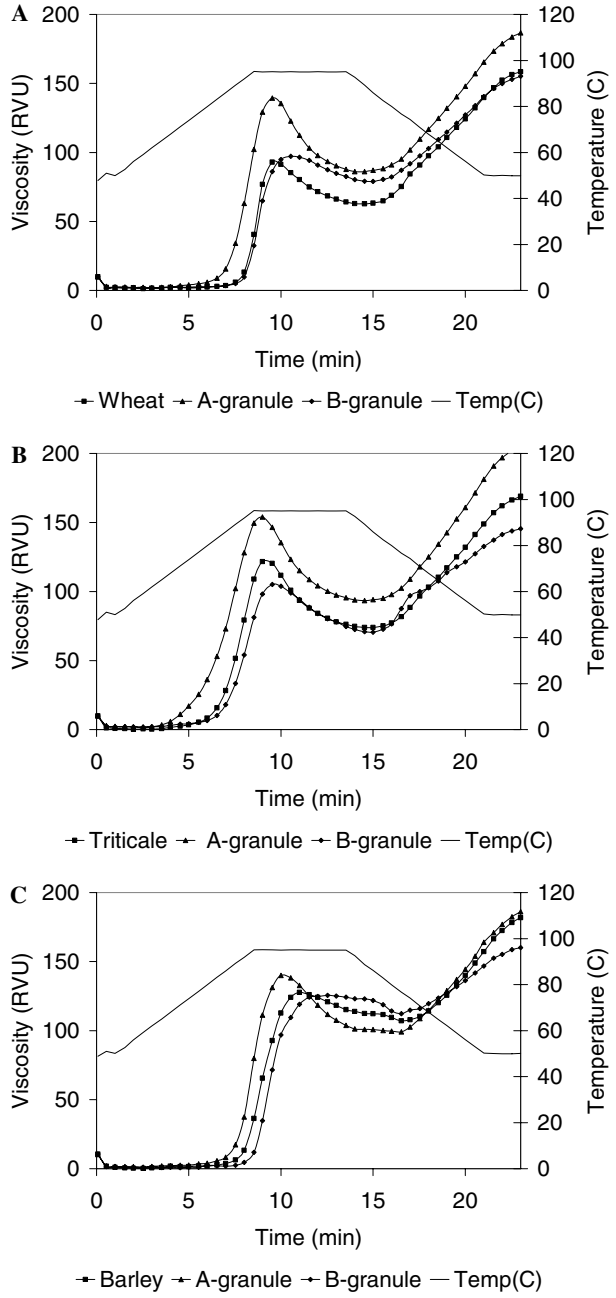

Fig. 5. Pasting profiles of (A) wheat, (B) triticale, and (C) barley starches measured by using a Rapid Visco-Analyzer.

After the gelatinized starches were stored at 4 °C for 7 days, the A-granules displayed greater retrogradation rates than did the B-granule counterparts (Table 4). This was attributed to the fact that the A-granules consisted of more amylose and more long branch-chains and lesser short branch-chains in the amylopectin than did the B-granules. The retrogradation rate of starch was inversely correlated with the proportion of short amylopectin branch-chains of DP 6–9 (Shi & Seib, 1992). The presence of lipids and phospholipids in the B-granules (Table 3) also retarded their retrogradation.

3.5. Pasting properties

Pasting curves of wheat, triticale, and barley starches are shown in Fig. 5, and the data are summarized in Table 5. The B-granule starch had higher pasting temperature (92.0, 84.7, and 94.3 °C for wheat, triticale, and barley, respectively) than did the A-granule starch (85.0, 76.1, and 88.0 °C, respectively) (Table 5). The A-granule starch had greater peak, final, breakdown, and setback viscosities than did the B-granule starch counterparts (Table 5). These results agreed with those reported by Sahlstrom et al. (2003) and Shinde et al. (2003). Pasting properties of starch are affected by starch granule size, amylose and lipid contents, and amylopectin structures. Amylopectin is primarily responsible for granule swelling, whereas amylose and lipid restrict the swelling (Tester & Morrison, 1990). The B-granule starch had more lipids than the A-granule starch, and lipids formed helical complexes with amylose, which restricted granule swelling. Thus, the B-granule starch developed lower peak viscosity at a higher pasting temperature. The setback viscosity of the A-granule starch, which reflected the network formation, was greater than that of the B-granule starch. This could be attributed to the fact that the A-granule starch consisted of more amylose and more long branch-chains of amylopectin than the B-granule starch.

To reveal whether the granular size of the starch or the chemical structures of the starch was more important in determining the pasting properties of wheat starch, we compared the pasting properties of the reconstituted wheat

Table 5
Pasting properties of wheat, triticale, and barley starches

Sample[a]	Pasting temperature (°C)	Viscosity (RVU)[b]				
		Peak	Hot paste	Breakdown	Final viscosity	Setback
Wheat	91.0	93.5	62.5	31.0	158.5	96.0
Wheat A-granule	85.0	139.8	85.9	54.0	186.8	100.9
Wheat B-granule	92.0	97.6	78.8	18.8	155.3	76.57
Triticale	82.1	123.0	73.8	49.2	169.1	95.4
Triticale A-granule	76.1	154.3	92.9	61.4	206.0	113.0
Triticale B-granule	84.7	105.2	70.4	34.8	145.5	75.1
Barley	91.5	127.8	106.6	21.2	181.9	75.3
Barley A-granule	88.0	140.3	98.8	41.6	186.3	87.5
Barley B-granule	94.3	125.9	112.3	13.6	160.2	47.8

[a] Starch sample suspensions were 8% (w/w, dsb).
[b] Measured in Rapid Visco-Analyzer units.

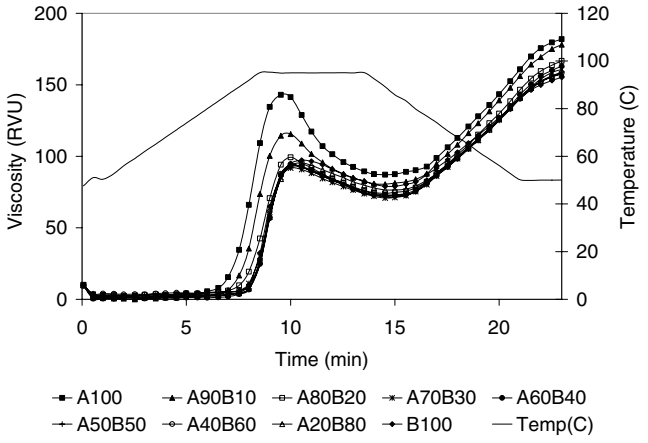

Fig. 6. Pasting profiles of reconstituted wheat starch containing different proportions of wheat A- and B-granules measured by using a Rapid Visco-Analyzer. A100 and B100 represent 100% of A- and B-granules, respectively. A90B10, A80B20, A70B30, A60B40, A50B50, A40B60, and A20B80 indicates that the contents of the A-granules are 90, 80, 70, 60, 50, 40, and 20%, respectively.

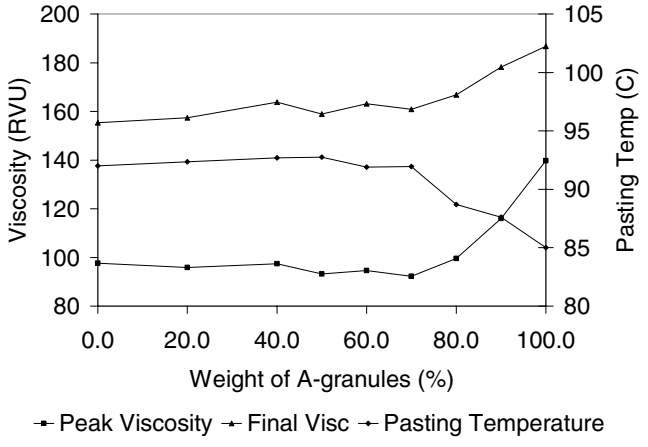

Fig. 7. Relationship between the A-granule content (by weight) and the peak viscosity (RVU), final viscosity (RVU), and pasting temperature (°C) of the reconstituted wheat starches measured by using a Rapid Visco-Analyzer.

starch that consisted of different proportions of A- and B-granules (w/w). We hypothesize that if the starch granule size plays the primary role controlling the pasting property, the pasting curve of the reconstituted starch should be correlated to the proportion of the A- and B-granules. Experimental results showed that the pasting properties of the reconstituted wheat starches were not changed when the A-granule content varied between 0% and 70% (Figs. 6, 7, and Table 6). Significant correlations, however, were found between parameters of the pasting properties and the content of the A-granules varying between 70% and 100%. The correlation coefficients between the pasting temperature, peak, and final viscosities and the content of wheat A-granules (70–100%), were −0.984, 0.974, and 0.994, respectively. The results showed that increasing the B-granule content from 0% to 30% significantly increased the pasting temperature and decreased the viscosity of the starch, but no further changes were found with more than 30% B-granules in the reconstituted starch. These results suggest that the composition of the starch, such as phospholipids present in the B-granules, rather than granule size, play the primary role controlling the pasting properties of the wheat starch.

3.6. Structure models of the A- and B-granules

Starches from different botanical origins have different characteristic granule shapes and morphology. This characteristic granule morphology is a result of different starch biosynthetic isozymes and different membranous structure and physical characteristic of amyloplasts present in different plants and organs (Jane et al., 1994). Gallant, Bouchet, and Baldwin (1997) proposed a blocklet model of the starch granule architecture. In this model, a blocklet contains several amorphous and crystalline lamellae. The blocklets range between 20 and 500 nm in diameter depending on the botanical source of the starch. The shape and structure of the starch crystallites in the blocklet are, however, determined by the structure and arrangement of amylopectin molecules.

It is known that the growth of a starch granule is by apposition (Yoshida, Fujii, Nikuni, & Maruo, 1958). The A- and B-granules, having disk and spherical shapes,

Table 6
Pasting properties of the reconstituted wheat starches with different proportions of the A- and B-granules

Sample[a]	Pasting temperature (°C)	Viscosity (RVU)[b]				
		Peak	Hot paste	Breakdown	Final viscosity	Setback
Wheat A100	85.0	139.8	85.9	54.0	186.8	100.9
Wheat A90B10	87.6	116.0	80.5	35.5	178.2	97.7
Wheat A80B20	88.7	99.6	73.9	25.7	166.8	92.9
Wheat A70B30	92.0	92.3	70.8	21.5	160.9	90.2
Wheat A60B40	91.9	94.6	72.0	22.6	163.1	91.1
Wheat A50B50	92.8	93.3	71.7	21.6	158.9	87.3
Wheat A40B60	92.7	97.4	73.8	23.7	163.8	90.1
Wheat A20B80	92.4	95.8	76.3	19.6	157.3	81.1
Wheat B100	92.0	97.6	78.8	18.8	155.3	76.6

[a] Starch sample suspensions were 8% (w/w, dsb). A100, A90B10, A80B20, A70B30, A60B40, A50B50, A40B60, A20B80, and B100 indicate that starch mixtures contain 100%, 90%, 80%, 70%, 60%, 50%, 40%, 20%, and 0% of wheat A-granules, respectively, with complement B-granules.
[b] Measured in Rapid Visco-Analyzer units.

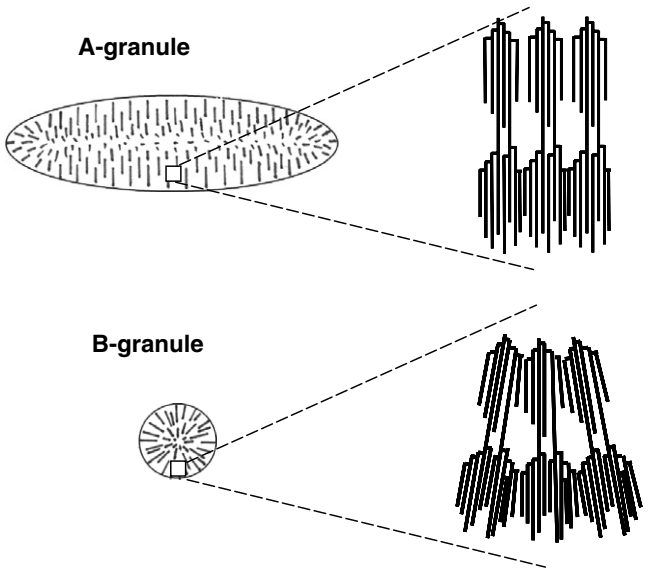

Fig. 8. Proposed granular and molecular structures of the A- and B-granules. The amylopectin branch-structure of the A- and B-granules were constructed on the basis of the molar ratios of short chains (A and B_1 chains) to long chains (B_2 and longer chains) of amylopectin branch-chain numbers of wheat A- and B-granule starches. Amylopectin molecules of the A-granule starch consisted of more long chains but lesser short chains, which displayed a cylindrical shape and better aligned parallel into a disk-shaped A-granule. Amylopectin molecules of the B-granule starch consisted of more short chains but lesser long chains, which displayed a cone shape. The cone-shaped molecules fitted in a spherical B-granule.

respectively, are likely to have amylose and amylopectin arranged differently in the granule. Optical map studies revealed that in a disk-shaped granule, such as the A-granules of wheat, triticale, and barley starch, the amylopectin molecules are oriented perpendicular to the equatorial plane of the disk-shaped granule and are aligned parallel (French, 1972). Amylopectin molecules that consisted of more B_2 chains but lesser short A and B_1 chains had a cylindrical shape, which can be better aligned in a parallel arrangement into a disk-shaped granule (Fig. 8). In contrast, amylopectin molecules that consisted of more short chains (A and B_1 chains) but lesser B_2 chains had a cone shape, which could be radially organized into a spherical granule (Fig. 8). The parallel arrangement of cylindrical-shaped amylopectin molecules in the disk-shaped A-granule resulted in an equatorial groove.

The cylindrical-shaped amylopectin molecules that are aligned parallel in the A-granules are expected to have greater percentage crystallinity than the cone-shaped amylopectin in the B-granules. These are supported by the X-ray diffraction results reported by Tang et al. (2002) that waxy barley A-granule starch has larger percentage crystallinity (36.6%) than does the B-granular starch (33.0%). The percentage crystallinity of the normal A-granule starch being smaller than that of the normal B-granule starch obtained in this study (Fig. 2) and in the study of Tang et al. (2002) is attributed to the fact that the amylose content of the normal A-granule starch is larger than that of the normal B-granule starch (Table 1) and amylose is amorphous in the granule, as discussed in Section 3.2.

4. Conclusion

Absolute amylose contents of wheat, triticale, and barley A-granule starches were greater than that of the B-granule starches. The disk-shaped A-granules consisted of amylopectin that had more long B_2 chains than did the spherical B-granules. Amylopectin molecules that have larger proportions of long B_2 chains and lesser short A and B_1 chains are likely to have a cylindrical-shape, which are aligned parallel into a disk-shaped A-granule. Amylopectin that consists of more short chains possesses a cone shape, which fits radially in a spherical granule. The B-granules had larger gelatinization-temperature ranges than did the A-granules. The B-granule starch retrograded more slowly than the A-granule starch because the B-granule consisted of less amylose and fewer long branch-chains in the amylopectin but more lipids. The B-granule starch had higher pasting temperature, less peak, breakdown, and setback viscosity than did the A-granule starch counterpart. Pasting properties of reconstituted wheat starch containing dif-

ferent proportions of the A- and the B-granules showed that the composition and chemical structures of the starch, instead of the size of the starch granules, determined the pasting properties of the starch. The results showed that the structures and properties of the A- and B-granule starches were distinct, which suggested that biosynthesis of the A- and the B-granules differed.

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