

Fine Structures of Amylose and Amylopectin from Large, Medium, and Small Waxy Barley Starch Granules

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ABSTRACT

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Amylose and amylopectin were prepared from large, medium, and small granule starches of classified waxy barley flour, and their fine structures were investigated. The amylose content had a wide distribution range (≈ 1.4 – 9.4%). Number-average degrees of polymerization (DP_n) of the amyloses were similar among the samples ($\approx 1,200$ – $1,300$). But number of chains per molecule (NC) decreased from the surface to the center (≈ 6 – 10 chains). DP_n of the amylopectins varied from 4,657 to 14,604; decreased in the order of large, medium, and small granules in same

fractions of the grain; and increased from the surface layer to the center. Longest chains (LC) were not found in any of the amylopectin molecules. The large amylopectin molecule had more long chains and fewer A chains than the small molecule. The amylose content had definite effects on the transition temperature range and crystal formation of the starch granules. There were positive correlations between DP_n of the amylopectin and relative crystallinity ($\gamma = +0.69$) and enthalpy value ($\gamma = +0.80$), respectively. These findings may help to elucidate biosynthesis mechanism of starch.

Starch differs in structure and physicochemical properties according to plant origin and variety (Naka et al 1985; Vasanthan and Bhatta 1996; Czuchajowska et al 1998; Klucinec and Thompson 1998; Sasaki and Matsuki 1998; Zeng et al 1998; Peng et al 1999). Wheat and barley starches are distinct from other starches in the distribution of granular size; that is, they have a bimodal distribution of large and small granules, referred to as A (large) and B (small) granules, respectively. Vasanthan and Bhatta (1996) and Peng et al (1999) separated A and B granules of starch from wheat endosperm and characterized physicochemical properties. Takeda et al (1999) prepared large, medium, and small starch granules from barley endosperm and characterized the structure of amylose and amylopectin. We showed that the ratio of large granules decreased from the surface layer to the center in waxy barley grain, and the ratio of medium and small granules increased. The physicochemical properties of the granules differed with granule size and between fraction. These findings suggested that the starch granules have different characteristics of the structure and components in the fractions of barley endosperm (Tang et al 2000).

Starches are divided into two fractions based on the difference of solubility to 1-butanol (Schoch 1942). The procedure was modified by other workers (Wilson et al 1943; Lansky et al 1949; Whistler and Doane 1961; Adkins and Greenwood 1969; Takeda et al 1986; Wang and White 1994; Klucinec and Thompson 1998). An intermediate material that precipitated with isoamyl alcohol and 1-butanol but not with 1-butanol alone is defined. The intermediate materials varied from 4 to 9% in normal and high amylose starches (Whistler and Doane 1961; Adkins and Greenwood 1969; Klucinec and Thompson 1998). In other words, amylose and amylopectin are the predominant fractions obtained by alcohol precipitation.

In this study, the amylose and amylopectin specimens from large, medium, and small granule starches of different fractions of waxy barley endosperm were separated and characterized. Correlations between the components and properties (Tang et al 2000) of the starch granules and among parameters of amylopectin structure were also discussed.

MATERIALS AND METHODS

Materials

The large, medium, and small starch granules isolated from classified waxy barley flour (*Hordeum vulgare* L. emed. Yonezawa No. 2, six-rowed, a product of Okayama) were used as described previously (Tang et al 2000). Flour fractions are indicated as A (100–90), B (90–80), C (80–70), D (70–60), E (60–50), F (50–40), G (40–30), and H (30–0) from the surface layer to the center of the grain (Tang et al 2000).

Crystalline *Pseudomonas* isoamylase and Shodex Standard P-82 were products of Hayashibara Biochemical Laboratories (Okayama). All chemicals were purchased from commercial suppliers.

Methods

Amylose and amylopectin specimens were fractionated and purified from the prepared starch granules following the procedure of Takeda et al (1986). From the large, medium, and small granules (10 g, dry weight) were produced 0.3–0.8, 0.2–0.8, and 0.1–0.9 g of amyloses, and 8.5–9.0, 8.4–9.1, and 8.0–9.0 g of amylopectins, respectively.

Iodine absorption spectra were measured following the methods of Takeda et al (1983). The amylose content and apparent content in starch were calculated from blue value (BV at 680 nm):

$$\text{Amylose content} = \frac{[\text{BV (starch)} - \text{amylopectin}]}{\text{BV (amylose)} - \text{amylopectin}} \times 100$$

$$\text{Apparent content} = \frac{[\text{BV (starch)}]}{\text{BV (amylose)}} \times 100$$

assuming the amylose BV = 1.2 (Takeda et al 1983).

Isoamylolysis of starch was followed the procedure of Hizukuri (1985). The number-average degrees of polymerization (DP_n) and the average chain length (CL) after isoamylolysis were determined by the method of Hizukuri et al (1981). The average number of chains per molecule (NC) = $[(DP_n/CL) - 1]$ (Hizukuri et al 1981; Suzuki et al 1981).

Gel filtration of the debranched starch was done by HPLC on two sequentially linked columns (TSK gel G3000SW + G2000SW, 7.5 mm \times 60 cm) maintained at 35°C. The elute was detected by Shimadzu RID-2A and the outputs of the detectors were recorded with a Shimadzu C-R6A. The elution solvent was 0.1M sodium acetate buffer (pH 6.2) containing 0.02% sodium azide, and the flow rate was 0.6 mL/min. A 200- μ L volume was injected through a Dismic-13JP type filter (0.2 μ m). Pullulans of fraction P-400, P-100, P-20, P-10, and P-5 were used as standards. The degree of polymerization (dp) of the collected fractions (0.6 mL) was determined following the method of Hizukuri et al (1981).

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RESULTS AND DISCUSSION

Iodine Absorption Spectra and Amylose Content

BV at 680 nm, λ_{\max} , and amylose content of defatted starches of large, medium, and small granules in the different fractions of waxy endosperm are shown in Table I. The values were 531–575 nm, 0.054–0.183, 1.4–9.4%, and 4.5–15.3%, respectively. The true amylose content was lower than the apparent content, which is consistent with Takeda et al (1999). The values of the λ_{\max} , BV, and true amylose content in large, medium, and small granules decreased from the surface layer to the center of the grain, respectively, the same as those of 12 waxy barley genotypes (Tester and Morrison 1992). There was a strong relationship between amylose content and λ_{\max} of starch ($\lambda_{\max} = 524.40 + 5.28$ amylose content, $\gamma = +0.98$, $n = 15$), which gave an extrapolated value of 524.4 nm (at 0 amylose content)

TABLE I
Absorbance of Starch-Iodine Complex and Amylose Content of Starches from Fractions of Waxy Barley Grain^a

Materials	λ_{\max} (nm) ^b	BV ^b	Amylose Content (%) ^c
B			
Large	565 ± 1.5	0.183 ± 0.006	8.5 (15.3)
Medium	573 ± 0.6	0.181 ± 0.004	9.0 (15.1)
Small	575 ± 3.5	0.175 ± 0.004	9.4 (14.6)
D			
Large	545 ± 1.2	0.124 ± 0.009	4.2 (10.3)
Medium	550 ± 0.9	0.119 ± 0.004	3.9 (9.9)
Small	552 ± 3.5	0.099 ± 0.004	4.5 (8.3)
F			
Large	540 ± 1.5	0.120 ± 0.004	4.0 (10.0)
Medium	549 ± 1.8	0.119 ± 0.002	3.7 (9.9)
Small	535 ± 3.5	0.069 ± 0.004	2.2 (5.8)
G			
Large	538 ± 0.3	0.120 ± 0.002	3.5 (10.0)
Medium	538 ± 1.5	0.108 ± 0.001	2.7 (9.0)
Small	532 ± 0.6	0.059 ± 0.001	1.6 (4.9)
H			
Large	538 ± 0.3	0.114 ± 0.003	3.5 (9.5)
Medium	538 ± 1.2	0.103 ± 0.001	2.6 (8.6)
Small	531 ± 0.3	0.054 ± 0.002	1.4 (4.5)

^a Values are means ± standard deviation of three separate measurements.

^b Blue value (BV) = absorbance at 680 nm; λ_{\max} = peak absorbance value over the range of wavelengths examined.

^c Amylose content (%) = [BV(starch — amylopectin)/BV (amylose — amylopectin)] × 100. Values in parentheses = apparent content (%) = [BV (starch)/BV(amylose)] × 100, assuming the amylose BV = 1.2.

for pure amylopectin, a little lower than 527 nm reported by Tester and Morrison (1992). MacGregor et al (1971) reported that the ratio of amylose and amylopectin was not constant throughout the growing season. Amylopectin is synthesized at a relatively faster rate than amylose during early stages of growth in normal barley. A study of starch biosynthesis by Martin and Smith (1995) indicated that granule-bound starch synthase (GBSS) synthesized the amylose component, whereas the amylopectin component was synthesized by soluble starch synthase (SSS) and starch branching enzyme (SBE). But the time at which each isoform of the biosynthetic enzymes is active and the level of activity differ among plant cultivars and within homologous tissue (Nelson et al 1978; Shannon and Garwood 1984; Dry et al 1992; Mizuno et al 1992, 1993). This suggests that, even within homologous endosperm, starch granules of different parts are not always synthesized simultaneously. Thus, these data may be useful to elucidate biosynthesis mechanism of starch.

Also, a higher positive correlation was found between λ_{\max} of the starches and the transition temperature range of the starch granules ($T_c - T_o = -75.79 + 0.16 \lambda_{\max}$, $\gamma = +0.89$, $n = 15$). The amylose content of the starches showed a negative and positive correlation with the relative crystallinity (relative crystallinity = $34.98 - 0.82$ amylose content, $\gamma = -0.75$, $n = 9$) and $T_c - T_o$ ($T_c - T_o = 7.68 + 0.86$ amylose content, $\gamma = +0.88$, $n = 15$). The relative crystallinity and transition temperature range, when extrapolated, were ≈35% and 7.7°C for the waxy starch granules at zero amylose content, respectively. Jenkins and Donald (1995) investigated the effect of varying amylose content on the internal structure of maize, barley, and pea starch species, and indicated that amylose disrupts the structure order within the amylopectin crystallites. Klucinec and Thompson (1999) investigated component interaction in retrogradation of dispersed starches and suggested that amylose-amylopectin interaction was influenced by the proportion of amylose-amylopectin and the chain lengths and the size distribution of amylopectin. Longer double helices should have a wider endotherm and temperature range (Klucinec and Thompson 1999). These results suggested that long double helices may be formed between amyloses or between amylose and amylopectin within the starch granule of the surface layer. However, there is no evidence of this.

Properties of Amylose Molecules

Properties of amylose molecules are given in Table II. The λ_{\max} values were 656–668 nm and were a little larger for the medium granules than large and small granules in the same fraction. The

TABLE II
Properties of Waxy Barley Amylose Molecules^{a,b}

Materials	λ_{\max} (nm)	BV	DP _n	CL	NC
B					
Large	656 ± 3.8	1.239 ± 0.009	1,200 ± 104	116	9
Medium	668 ± 1.5	1.253 ± 0.028	1,260 ± 111	115	10
Small	661 ± 2.1	1.238 ± 0.060	1,290 ± 96	116	10
D					
Large	664 ± 2.5	1.239 ± 0.038	1,290 ± 63	151	8
Medium	668 ± 1.0	1.236 ± 0.040	1,330 ± 63	158	7
Small	662 ± 3.0	1.230 ± 0.018	1,250 ± 48	149	7
F					
Large	660 ± 1.5	1.238 ± 0.025	1,330 ± 15	176	7
Medium	666 ± 3.2	1.229 ± 0.025	1,330 ± 148	175	7
Small	662 ± 0.6	1.220 ± 0.058	1,280 ± 111	177	6
G					
Large	659 ± 1.8	1.242 ± 0.028	1,290 ± 60	220	5
Medium	667 ± 1.5	1.239 ± 0.057	1,300 ± 92	191	6
Small	660 ± 2.5	1.240 ± 0.039	1,210 ± 102	172	6
H					
Large	658 ± 1.8	1.232 ± 0.016	1,280 ± 69	193	6
Medium	667 ± 2.1	1.236 ± 0.021	1,230 ± 136	188	6
Small	662 ± 0.9	1.222 ± 0.005	1,270 ± 81	170	6

^a Blue value (BV) = absorbance at 680 nm; λ_{\max} = peak absorbance value over the range of wavelengths examined; DP_n = number-average degrees of polymerization; CL = average chain-length; NC = number of chains per molecule.

^b Values are means ± standard deviation of six separate measurements.

values generally agreed with those of previous reports (Takeda et al 1986; Schulman et al 1995; Takeda et al 1999). BV for all amyloses was approximately the same (1.220–1.253). The values were lower than for normal barley (Schulman et al 1995; Takeda et al 1999) and rice (Takeda et al 1986). DP_n of the amyloses was 1,200–1,330, and similar between the samples. Schulman et al (1995) indicated that DP_n of the amyloses in normal and *shx* barley (*H. vulgare*) were 1,120 and 1,230, respectively. Takeda et al (1999) reported 1,610–1,900 for normal barley of *H. distichum*. CL of the amyloses was 115–220, similar to normal (210) and *shx* (230) barley of *H. vulgare* (Schulman et al 1995), except at the surface layer, but not normal barley (344–358) of *H. distichum* (Takeda et al 1999) and normal rice (Takeda et al 1986). NC of the amyloses was ≈6–10 chains. It decreased from the surface layer to the center of the grain. This is similar to results reported previously (Schulman et al 1995; Takeda et al 1999) for the center of the grain. Thus, the findings indicated that the structures of the amyloses in waxy barley endosperm differed among the granule sizes and among fractions. However, further study is needed on the ratio of the branched molecules and distribution of molecular weight.

Gel-Permeation Chromatography of Debranched Starch

When preparations of starch debranched by isoamylase were fractionated on two columns, TSKG3000SW + TSKG2000SW, one minor peak and three major peaks of refractive index (Ri) response were obtained (Fig. 1). The major peaks, considered to be of amylopectin origin, were divided into three fractions, F1 to F3. F1 was the long chain fraction, and F2 and F3 were the short chain fractions. The profiles of the long and short chain fractions showed a few differences among fractions of grain. The minor peak was of the amylose origin, and the average dp of the peaks was 1,300–1,400 residues and corresponded to the dp_n of amylose (Table II). The area of the peaks decreased from the surface layer to the center of the grain, corresponding to the tendency for amylose content to decrease (Table I). These results reflected the different characteristics of the branched amylose molecule among different fractions (Table II).

Structure of Amylopectin

Properties of the amylopectin molecules are shown in Table III. The λ_{max} of the amylopectins ranged from 527 to 537 nm and decreased in the order of large, medium, and small granules in the same fraction, but showed no significant difference among fractions. They were lower, however, than for normal barley (Schulman et al

1995; Takeda et al 1999), normal wheat (Hizukuri and Maehara 1990), and normal rice (Takeda et al 1986). They agreed with the extrapolated values (527–529 nm) for pure amylopectin (Tester and Morrison 1992), although they were a little higher for the large granules. The BV for the amylopectins was 0.038–0.085. The BV of the small granules was the lowest in the same fraction. The values were lower than those of normal barley (Schulman et al 1995; Takeda et al 1999) and wheat amylopectin (Hizukuri and Maehara 1990), but similar to those of japonica rice (Takeda et al 1987). The CL of the amylopectins in fractions was similar (17–19). The value for small granules was lower than for large and medium

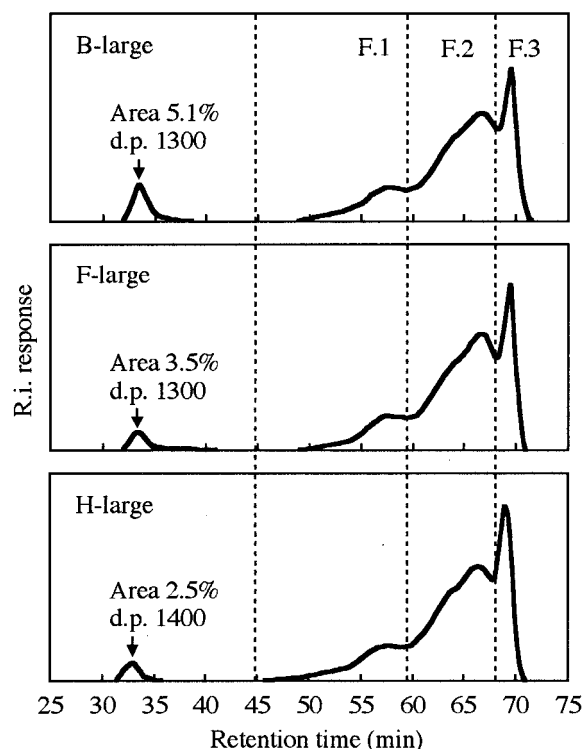


Fig. 1. Gel-permeation HPLC of isoamylase-debranched starches. Column: TSKG3000SW + TSKG2000SW (7.5 × 60 cm) at 35°C; flow rate: 0.6 mL/min; buffer: 0.1M sodium acetate buffer (pH 6.2) containing 0.02% sodium azide.

TABLE III
Properties of Waxy Barley Amylopectin Molecules^{a,b}

Materials	λ _{max} (nm)	BV	DP _n	CL	NC
B					
Large	532 ± 0.3	0.085 ± 0.001	8,171 ± 744	19	430
Medium	530 ± 3.2	0.077 ± 0.004	7,043 ± 758	18	391
Small	527 ± 0.6	0.065 ± 0	4,657 ± 420	18	254
D					
Large	535 ± 2.9	0.075 ± 0.001	9,122 ± 710	19	479
Medium	530 ± 1.2	0.075 ± 0.002	7,167 ± 269	18	397
Small	528 ± 0.9	0.046 ± 0.002	5,761 ± 586	17	339
F					
Large	537 ± 0.9	0.074 ± 0.002	11,481 ± 998	19	604
Medium	529 ± 0.9	0.077 ± 0.002	8,553 ± 502	18	420
Small	527 ± 0.3	0.043 ± 0	6,675 ± 351	17	393
G					
Large	536 ± 0.3	0.079 ± 0.001	14,325 ± 746	19	754
Medium	529 ± 0.9	0.077 ± 0.003	12,019 ± 561	18	668
Small	527 ± 0.6	0.040 ± 0.002	7,059 ± 293	17	415
H					
Large	537 ± 0.9	0.073 ± 0.001	14,604 ± 399	19	769
Medium	532 ± 1.5	0.073 ± 0.003	12,010 ± 534	17	706
Small	527 ± 0.0	0.038 ± 0.001	6,985 ± 270	17	411

^a Blue value (BV) = absorbance at 680 nm; λ_{max} = peak absorbance value over the range of wavelengths examined; DP_n = number-average degrees of polymerization; CL = average chain-length; NC = number of chains per molecule.

^b Values are means ± standard deviation of six separate measurements.

granules in the same fraction. The values for large and medium granules corresponded to those of previous reports (Schulman et al 1995; Takeda et al 1999). However, DP_n of the amylopectins had a wider range (4,657 to 14,604) and decreased in proportion to granule size in the same fraction and increased from the surface layer to the center of grain. The DP_n of the large and medium granules were greater than for normal barley (Schulman et al 1995) but similar to the values for japonica rice (Takeda et al 1987). DP_n of the small granules was similar to that of *s/hx* mutation of barley (7,800) (Schulman et al 1995) or indica rice (4,700–5,800) (Takeda et al 1987). The NC of the amylopectins also had a wide range (254–769) and showed a tendency similar to the DP_n of the amylopectins. The relative crystallinity and enthalpy value of the starch granules (Table IV) showed positive correlations with DP_n of the amylopectins ($DP_n = -10348 + 662.08$ relative crystallinity, $\gamma = +0.69$, $n = 9$; $DP_n = 333.97 + 1048.30$ enthalpy, $\gamma = +0.80$, $n = 15$). Gelatinization of starch involves melting of double helices and loss of crystalline. (Cooke and Gidley 1992). Thus, these data suggested the large amylopectin molecules formed more and stronger crystalline networks than the small molecules within the starch granules.

When the amylopectins debranched by isoamylase were fractionated on the column of TSKG3000SW + TSKG2000SW, the

profiles were similar to those of the debranched starches, except that the minor peaks were not present. Accordingly, the peaks were fractionated into three fractions, F1 to F3 (Fig. 2). The longest chain (LC) was not found in waxy barley amylopectin, as in previous reports (Hizukuri et al 1989; Schulman et al 1995). The result suggested that this was probably a general phenomenon. When the debranched amylopectins are fractionated according to Hizukuri (1986), F1 corresponds with long chains (B2, B3) that link clusters and make large molecules. F2 and F3 were short chains, B1 and A chains, respectively. However, the profiles of the short chain fractions differed from those reported previously (Hizukuri 1986; Schulman et al 1995; Takeda et al 1999) probably because of differences in column, solvent, and flow rate. However, this does not influence comparison between the samples because they were examined under the same conditions.

TABLE IV
Properties of Starch Granules^a

Samples	$T_c - T_0^b$	Enthalpy ΔH (J/g)	Relative Crystallinity ^c (%)
B			
Large	13.4	7.6	28.6
Medium	16.1	6.4	29.0
Small	17.7	4.6	23.7
D			
Large	11.0	9.8	nd ^d
Medium	10.3	7.7	nd
Small	11.2	5.3	nd
F			
Large	9.9	12.6	34.7
Medium	10.2	10.3	33.9
Small	11.6	8.3	32.5
G			
Large	10.0	11.8	nd
Medium	8.8	8.4	nd
Small	10.5	6.2	nd
H			
Large	9.6	10.8	34.3
Medium	8.9	8.4	31.1
Small	10.1	6.3	30.8

^a Tang et al (2000).

^b Range from onset to conclusion temperatures.

^c Ratio of areas of crystalline and amorphous regions of X-ray diffractograms by the method of Hermans (Nara et al 1978).

^d Not determined.

TABLE V
Properties of Chain-Length Distribution in Waxy Barley Amylopectins^{a,b}

Samples	F1			F2			F3			SF/LF		
	W%	M%	DP	W%	M%	DP	W%	M%	DP	SF DP	W	M
B												
Large	24.5	9.4	40.8	60.9	61.1	16.7	14.6	29.5	8.6	14	3.1	9.6
Medium	21.6	8.7	40.9	62.2	60.7	16.1	16.2	30.6	10.2	14	3.6	10.5
Small	20.9	7.5	40.1	57.5	60.5	15.0	21.6	32.0	9.2	13	3.8	12.3
F												
Large	23.9	9.2	40.5	60.8	59.8	17.1	15.3	31.0	9.3	14	3.2	9.9
Medium	20.1	8.6	40.1	59.5	56.9	17.2	20.4	34.5	10.1	14	4.0	10.6
Small	19.5	7.8	40.8	58.9	55.5	17.3	21.6	36.7	9.6	14	4.1	11.8
H												
Large	19.7	8.8	39.3	59.4	59.8	14.8	20.9	31.4	9.5	13	4.1	10.4
Medium	19.1	8.1	39.3	60.0	59.9	14.6	20.9	32.0	9.2	13	4.2	11.3
Small	21.5	7.6	40.4	58.8	57.5	15.9	19.7	34.9	8.6	13	3.7	12.2

^a Values are means of two separate measurements.

^b F = fraction; W = weight; M = molar; DP = average degrees of polymerization; SF = short-chain fraction (F2 + F3); LF = long-chain fraction (F1).

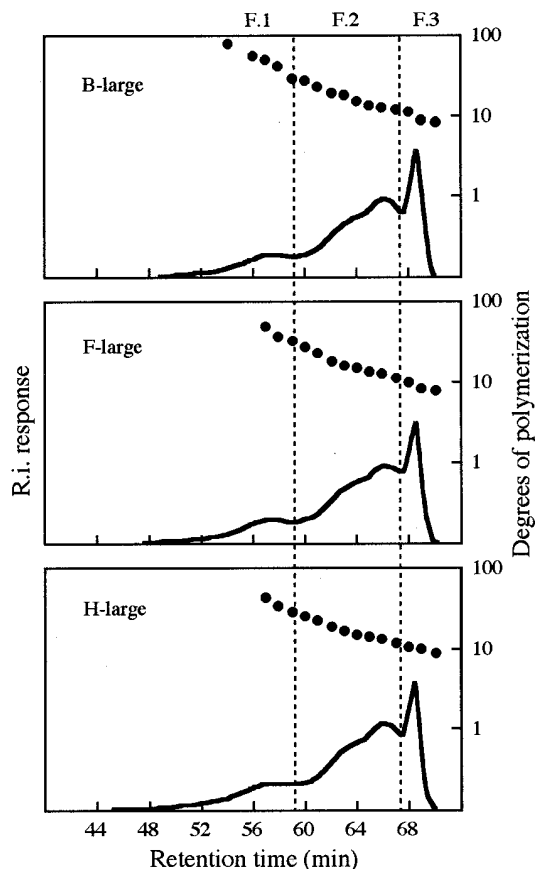


Fig. 2. Gel-permeation HPLC of isoamylase-debranched amylopectins from waxy barley starches; differential refractometry (—), degrees of polymerization (●).

The properties of CL distribution of the amylopectins are summarized in Table V. The molar proportion of F1 was 7.5–9.4% and decreased against the decrease of granule size in the same fraction. The dp was similar to the values of other reports (Schulman et al 1995; Takeda et al 1999). In F2, the molar proportion tended to decrease with granule size in the same fraction and be similar among fractions. The dp showed larger values in the intermediate layer (F-fraction) of the grain (≈ 17 residues). The molar proportion of F3 tended to increase with the decrease of granule size. The average dp of short chains in the H-fraction of the center was one residue smaller (≈ 13 residues) than in the B- and F-fractions (≈ 14 residues). The molar ratio of short to long chain increased in the order large, medium, and small granules in same fractions and was similar among fractions of the grain. The DP_n of the amylopectins showed a low positive and negative correlation with the molar percentage of the long chain fraction ($DP_n = -10297 + 2283.40 F1$, $\gamma = +0.50$, $n = 9$) and with the molar ratio of the short chain fraction to long chain fraction ($DP_n = 26972 - 1648.80 SF/LF$, $\gamma = -0.52$). The BV of the amylopectins correlated positively and negatively with the molar percentage of F2 ($F2 = 53.20 + 64.21 BV$ of amylopectin, $\gamma = +0.69$, $n = 9$) and F3 ($F3 = 40.40 - 117.41 BV$ of amylopectin, $\gamma = -0.81$, $n = 9$). These data indicated that the large molecule had more long chains and fewer A chains than the small molecule in waxy barley amylopectin. The short A chains (≤ 9 residues) may disrupt crystal formation.

CONCLUSIONS

The present results indicated that the content and structure of amylose and amylopectin differed with granule size and fraction in waxy barley endosperm starches. The amylose content appeared to cause definite effects on the transition temperature range and crystal formation of the starch granules. The large amylopectin molecules had more long chains and fewer A chains than small amylopectin molecules, and could form more and stronger crystalline networks within the starch granules. It may be better to investigate the quantitative correlation using starches from different parts of the grain because the grains grow in the same environment of temperature and soil. These correlations must be investigated further. These findings also may help to elucidate the biosynthesis mechanism of starch.

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