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Structures of large, medium and small starch granules of barley grain

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Abstract

The structure of amylose and amylopectin in large (L), medium (M) and small (S) starch granules fractionated from barley grain was examined. The L, M and S granules were 20, 7.5 and 2.3 μm in average diameter, and accounted for 79, 11 and 10% (w/w) in yield, respectively. The amylose content in L and M granules was 25.4 and 24.7%, respectively, higher than that (20.3%) in S granules. L and M granules had a smaller amylose (number average DP, $DP_n = 1640$ and 1610) with a smaller number of chains (NC = 5.6 and 4.9) than S granules ($DP_n = 1900$, NC = 7.2). The amylopectins from all the granules resembled each other in structure; they showed similar iodine affinity (0.60–0.75 g/100 g), average chain length (19–20), β-amylolysis limit (55–56%), and chain length distribution although the distribution of short side-chains (DP = 6–37) and the phosphorus content slightly differed. These findings suggest that the regulation or genetic control for the synthesis of S granules differs from that for L and M granules.

Keywords: Barley; Starch; Amylose; Amylopectin

1. Introduction

The analytical methods recently developed for starch show that the structure of starch differs with the plant origin and its variety (Takeda, 1993; Hizukuri, 1996). Cereal starches generally have a smaller amylose and an amylopectin with a higher amount of very long and short side-chains than root and tuber starches. Rice and maize amyloses are composed of a smaller number of chains per molecule than wheat and barley amyloses, and their amylopectins are different in the distribution of short side-chains. Amylomaize amylopectins are less branched than normal maize amylopectins (Takeda and Preiss, 1993; Takeda et al., 1993), and indica rice amylopectins have a larger amount of the very long chains than japonica rice amylopectins (Takeda et al., 1987a). On the other hand, wheat and barley starches are distinct from other starches in the distribution of granular size, that is, the bimodal distribution of large and small granules, referred to as A- and B-granules, respectively. The structure of these starch granules has been examined (Bathgate and Palmer, 1972; MacGregor and Ballance, 1980; MacGregor and Morgan, 1984; Naka et al., 1985; Kang et al., 1985a, 1985b), but not in detail, especially the fractionated amylose and amylopectin. We here prepared large, medium and small granules from barley grain by differential sedimentation and characterized the structure of their amylose and amylopectin.

2. Experimental

2.1. Materials

The large (L), medium (M) and small (S) starch granules from barley grain (Harunanijo, a two-rowed variety, *Hordeum distichum* L., a product of Kagoshima) were isolated as follows. The grain was steeped in 0.2% NaOH for 2 days in a cold room, and homogenized in a home blender (Takeda et al., 1988b). The homogenate was squeezed through cotton cloth and then filtered with 100-, 200- and 400-mesh sieves, successively. The starch was washed with 0.2% NaOH by centrifugation until no biuret reaction occurred, and washed with water. The starch was fractionated into L, M and S granules using 1 L cylinders by differential sedimentation: 2 h precipitate, 2–20 h precipitate, and 20 h supernatant, respectively. The fractionation was repeated four to five times on each fraction to be refined. L, M and S granules were 202, 28 and 25 g, respectively, from 600 g of grain, and the whole starch of 186 g was obtained from 400 g of grain.

The starch was fractionated into amylose and amylopectin as described previously (Takeda et al., 1986). L, M and S

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Table 1
Properties of the starch granules

Granule	Yield	Size	Iodine affinity (IA, g/100 g)				Amylose content[a]
	(%)	(μm)	Defatted (*A*)	Not defatted (*B*)	*A* − *B*	(*A* − *B*)/*A*	(%)
Whole	100	–	5.55	3.57	1.98	0.36	25.0 (27.8)[b]
Large	79	20.0 ± 3.9	5.63	4.01	1.62	0.29	25.4 (28.2)
Medium	11	7.5 ± 2.9	5.45	3.53	1.92	0.35	24.7 (27.3)
Small	10	2.3 ± 0.7	4.61	3.10	2.51	0.54	20.3 (23.1)

[a] Calculated by the equation: [(IA)starch − (IA)amylopectin]/[(IA)amylose − (IA)amylopectin] × 100, where the IA of amylose and amylopectin is from Tables 2 and 3.
[b] Apparent content, calculated by the equation: [(IA)starch]/[(IA)amylose] × 100, assuming the amylose IA to be 20.

granules (10 g dry weight) produced 2.3, 2.2 and 1.8 g of amylose and 7.3, 7.3 and 7.7 g of amylopectin, respectively. The whole starch of 10 g yielded 2.2 g of amylose and 7.3 g of amylopectin. Gel-permeation chromatography (Takeda et al., 1984) and HPLC with a low-angle laser-light scattering photometer (Hizukuri and Takagi, 1984) revealed that all amylose specimens were free of amylopectin.

The β-limit dextrin (β-LD) from each amylose was prepared from β-amylolyzate by gel-permeation chromatography on Bio-Gel P-4 (Takeda et al., 1987b). Sweet potato β-amylase was recrystallized from aqueous ammonium sulfate to improve its stability on storage (Takeda and Hizukuri, 1969). Crystalline *Pseudomonas* isoamylase was the product of Hayashibara Biochemical Laboratories Inc. (Okayama).

2.2. Analytical methods

Starch granule diameter was estimated by averaging the dimensions of more than 100 random granules from three SEM micrograms for each granule type. The iodine affinity (IA g/100 g) was determined at 25°C by automated amperometric titration (Takeda et al., 1987a). The blue value, limiting viscosity number ([η] mL/g, 1 M KOH, 22.5°C), number- (DP_n) (Hizukuri et al., 1981) and weight- (DP_w) (Hizukuri and Takagi, 1984) average degrees of polymerization, average chain-length (CL) (Hizukuri and Osaki, 1978; Hizukuri et al., 1981), and the β-amylolysis limit (β-AL) (Suzuki et al., 1981) were determined as described previously. The average number of chains per molecule (NC) was DP_n/CL. The chain distribution of amylopectin after isoamylolysis was determined by gel-permeation HPLC with a low-angle laser-light scattering photometer (Hizukuri, 1986) and anion-exchange chromatography with pulsed amperometric detection (Hanashiro et al., 1996). Carbohydrates were determined by the phenol–H_2SO_4 method (Dubois et al., 1956).

3. Results and discussion

3.1. Size and amylose content of the fractionated granules

The L, M and S starch granules had average diameters of 20, 7.5 and 2.3 μm and accounted for 79, 11 and 10% by weight, respectively (Table 1). The values differed from those obtained by sieving of barley starch through a nylon screen which yielded 89 and 11% (w/w) of large (about 25 μm) and small (about 5 μm) granules, respectively (Bathgate and Palmer, 1972). However, these findings

Table 2
Properties of amylose and its beta-limit dextrin (β-LD)

Starch	Whole		Large		Medium		Small	
granule	Amylose	β-LD	Amylose	β-LD	Amylose	β-LD	Amylose	β-LD
IA (g/100 g)	20.1	18.8	20.1	18.6	20.2	18.9	19.8	18.6
Blue value	1.63	1.51	1.63	1.53	1.61	1.56	1.63	1.54
λ_{max} (nm)	664	661	666	663	665	662	665	664
[η] (mL/g)	344	–	349	–	344	–	358	–
Number-average DP, DP_n	1570	1440	1640	1520	1610	1600	1900	1810
Weight-average DP, DP_w	5580	5930	5840	6170	5540	5870	5810	6270
DP_w/DP_n	3.56	4.24	3.56	3.98	3.44	3.67	3.06	3.46
Average chain-length, CL	315	120	295	115	330	155	265	110
No. of chains per molecule, NC	5.0	12.0	5.6	13.2	4.9	10.3	7.2	16.5
β-Amylolysis limit, β-AL (%)	76		75		76		73	
Molar fraction (% mol) of:								
Branched molecule	35		35		42		40	
Linear molecule	65		65		58		60	

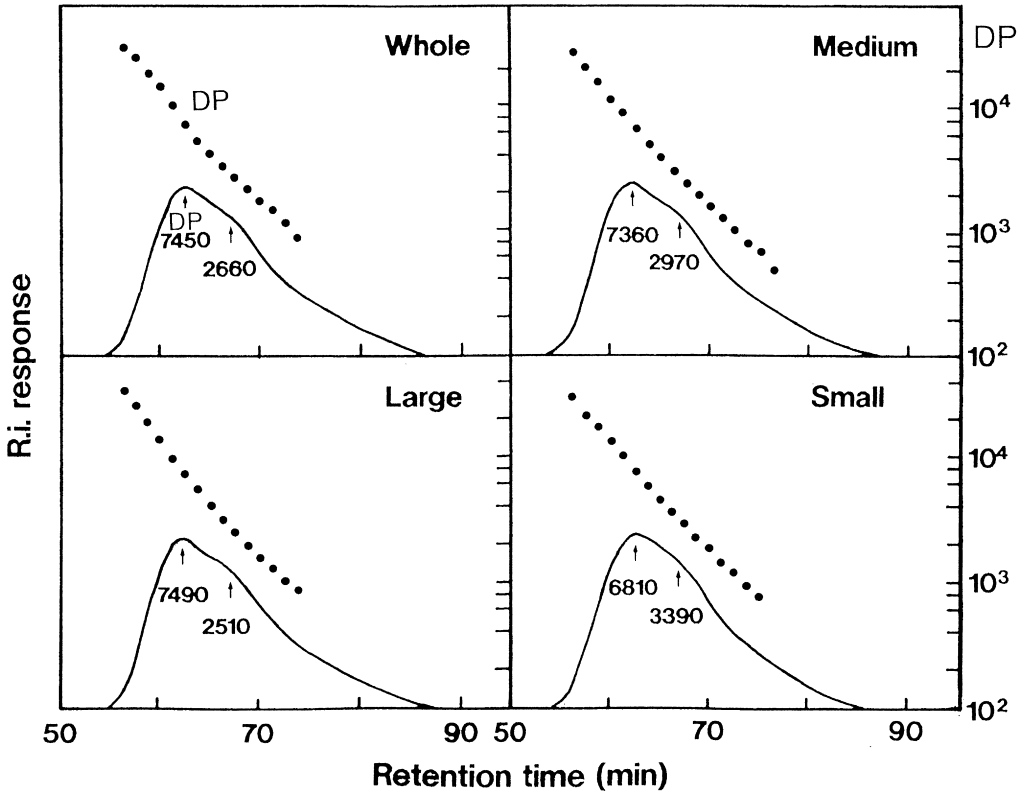

Fig. 1. Gel-permeation HPLC of the amyloses from the whole and fractionated granules: ———, differential refractometry; ●, DP.

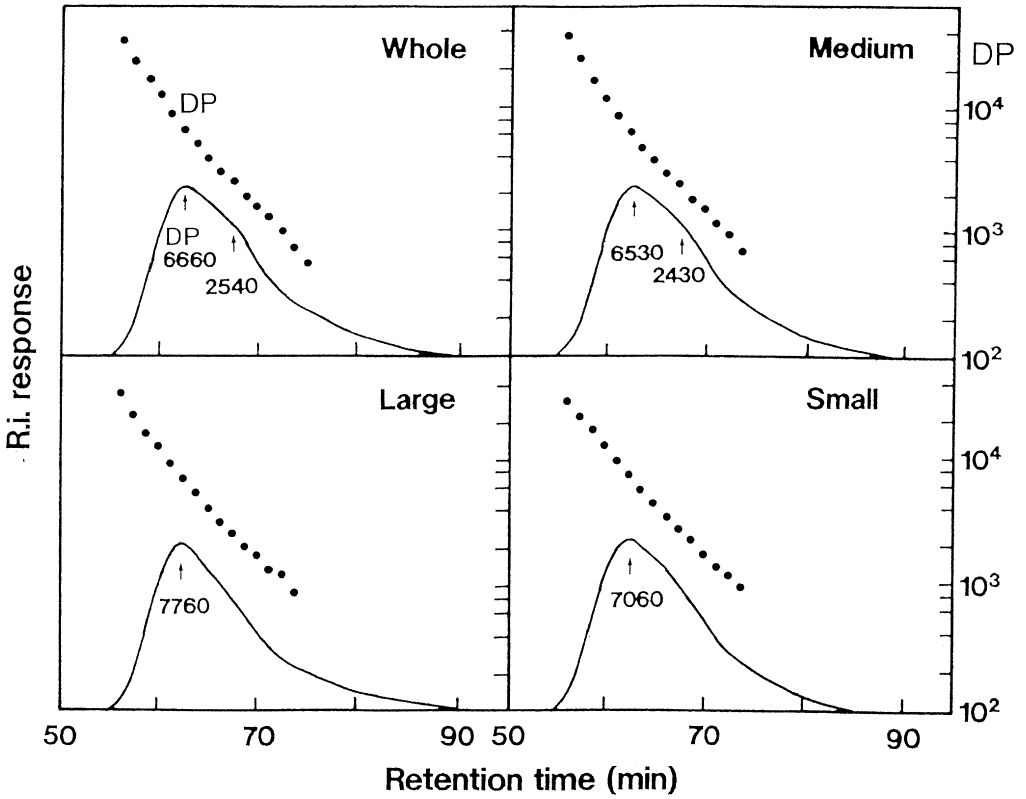

Fig. 2. Gel-permeation HPLC of the β-limit dextrins from the amyloses of the whole and fractionated granules: ———, differential refractometry; ●, DP.

Table 3
Properties of amylopectins

Starch granule	Whole	Large	Medium	Small
IA (g/100 g)	0.69	0.71	0.60	0.75
Blue value	0.11	0.11	0.10	0.10
λ_{max} (nm)	541	544	539	540
[η] (mL/g)	155	163	157	144
CL				
Smith degradation	18	19	18	18
Isoamylolysis	19	20	19	19
β-AL (%)	55	55	55	56
Phosphorus (μg/g)				
Organic, P_o	15	14	19	27
Linked to C-6, P-6	4	4	10	7
P-6/P_o (%)	27	24	53	26

indicated that S granules comprised a minor component by weight but major component by number (Bathgate and Palmer, 1972; Morrison et al., 1986). The amylose content, calculated from IA values of defatted starch, amylose and amylopectin, was 20.3% for S granules, being about 5% lower than those of L and M granules and similar to the previous values (small granules (1–2 μm) 20.4%, large granules (10–20 μm) 23.6%) (MacGregor and Ballance, 1980). These contents were about 3% lower for each granule than the apparent amylose contents, which were calculated without consideration of IA of amylopectin, due to a relatively higher IA of the amylopectins. S granules showed a larger difference in IA before and after defatting than L and M granules, and had a higher proportion (54%) of amylose forming a complex with lipids than the other granules (29–35%).

The whole granule had an amylose content of 25%, which was similar to L granules. This value was lower than those for the other barley varieties (27.5–28.2%) (Schulman et al., 1995), higher than those for rice (16–19%) (Hizukuri et al., 1989) and normal maize (19–21%) (Takeda et al., 1988a; Takeda and Preiss, 1993), and in the range for wheat (22–27%) (Shibanuma et al., 1994, 1996).

3.2. Amylose structure

The amyloses from L, M and S granules were similar in their values of IA, blue value, λ_{max} and β-AL (Table 2). The

Table 4
DP_w and amounts of carbohydrate of chains of amylopectins after isoamylolysis

Starch	DP_w					% (w/w) of total				
granule	A	B_1	B_2	B_3	A–B_3	A	B_1	B_2	B_3	LC
Whole	12	23	51	205	26	41	36	20	1	2
Large	12	24	51	230	26	42	35	20	1	2
Medium	12	23	49	205	25	43	35	19	1	2
Small	13	23	50	200	26	43	34	19	1	3

The subfractions, A, B_1–B_3, and LC, are shown in Fig. 3.

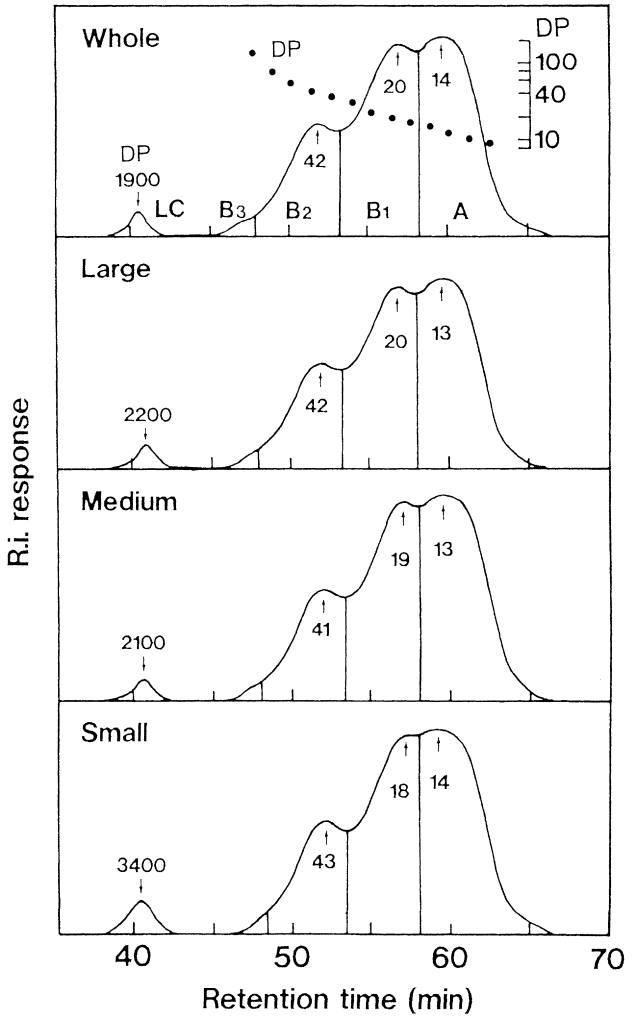

Fig. 3. Gel-permeation HPLC of the amylopectins from whole and fractionated granules after isoamylolysis: ———, differential refractometry; ●, DP.

DP_n and [η] values indicated that S-amylose (DP_n = 1900) was larger than L- and M-amyloses (DP_n = 1640 and 1610, respectively), although S-amylose had a similar DP_w to L-amylose. S-Amylose had a smaller CL (265) and a larger NC (7.2) than L- and M-amyloses (CL = 295 and 330; NC = 5.6 and 4.9). Gel-permeation chromatograms (Fig. 1) indicated that all the amyloses showed a similar elution profile with a peak (DP = 6810–7490) and a shoulder (DP = 2510–3390). However, S-amylose showed the smallest DP_w/DP_n ratio, suggesting that it had the narrowest DP distribution among them. The β-LD derived from the branched molecule of the amyloses (Takeda et al., 1987b) showed similar values of IA, blue value and λ_{max} and a similar DP distribution (Table 2 and Fig. 2). However, the branched molecule of S-amylose might be the largest and have the narrowest DP distribution judging from the DP_n, DP_w and DP_w/DP_n values of the β-LD. The branched molecule in S-amylose was composed of 16.5 chains on average, and was more highly branched than those in

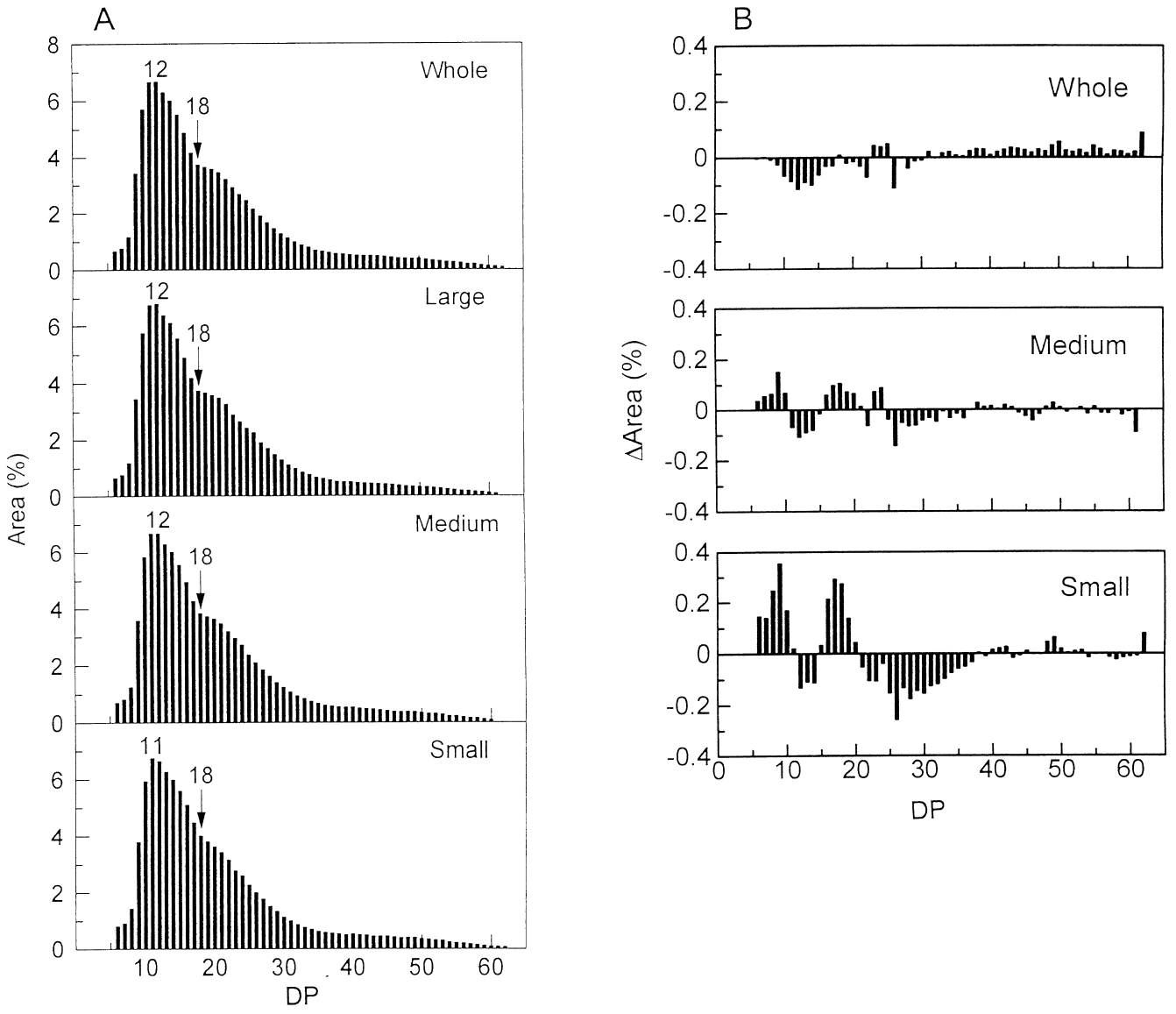

Fig. 4. Chain-length distributions of the amylopectins from the whole and fractionated granules (A), and differences in chain-length distribution against the amylopectin from L granules (B).

L- and M-amyloses. The branched molecule in M-amylose might have a longer inner chain than the others because the β-LD had a higher CL value. In each amylose, the branched molecule was the minor component by number (36–42 mol%), and the linear molecule was the major component. Thus, the structure of amylose differed between the different sizes of starch granule.

The amylose from the whole granule was similar to L-amylose in structure (Table 2 and Fig. 1). The amylose had a DP_n of 1570 and DP_w of 5580, which is larger than those of other barley varieties (DP_n = 1120–1230; DP_w = 4470–4610) (Schulman et al., 1995), but had a similar NC of 5.0. The amylose differed in size from those for rice (DP_n = 1100–1110, DP_w = 2750–3420) (Hizukuri et al., 1989), maize (DP_n = 830–960, DP_w = 2410–2680) (Takeda et al., 1988a; Takeda and Preiss, 1993) and wheat (DP_n = 830–1570, DP_w = 2360–5450) (Shibanuma et al., 1994, 1996), and had more branches than rice (NC = 3.5–4.2) and maize (2.4–2.9) amyloses, but less branches than wheat amylose (5.5–7.6). The branched molecule of the amylose was composed of 12.0 chains on average, being higher than those for rice (8.5, 9.0) and maize (5.3), but less than that for wheat (12.9–20.7).

3.3. Amylopectin structure

The analytical data in Tables 3 and 4, and Fig. 3 implied that the amylopectins from L, M and S granules generally resembled each other in structure. The amylopectins showed similar values of IA (0.60–0.75), blue value (0.10–0.11), λ_{max} (539–544 nm), CL (19) and β-AL (55–56%). They had similar amounts of very long (LC) and A–B_3 chains, which

were fractionated according to Hizukuri (1986), and had similar peak DP and DP_w values for $A–B_3$ chains. However, these amylopectins differed slightly in some fine structures. The distribution of short side-chains (Fig. 4) showed that S-amylopectin had a higher amount of chains with DP = 6–9 and DP = 16–19 and a smaller amount of chains with DP = 20–37 than L- and M-amylopectins. The [η] values suggested that S-amylopectin was smaller than M- and L-amylopectins. S-Amylopectin had a slightly higher phosphorus content than the other amylopectins. The proportion of phosphorus linked to C-6 of the glucose residue was about 25% for L- and S-amylopectins, and 53% for M-amylopectin.

The amylopectin from the whole granule had a structure resembling L-amylopectin and appeared similar to the amylopectins from other varieties of barley (Schulman et al., 1995). The amylopectin of barley appeared to be similar to rice, wheat and maize amylopectins in CL and phosphorus content. Among the amylopectins from these origins, barley amylopectin was similar to wheat amylopectin, especially in the distribution of side chains showing individual peaks of B1 and A chains, while the other amylopectins showed B1 and A chains as a peak.

The present results indicated that S granules, being a major component by number, differed from L granules in terms of the amylose content and structure of both amylose and its branched molecule, and also some structural properties of amylopectin. M granules resembled L granules rather than S granules in structure. These findings suggest that the regulation or genetic control for the synthesis for S granules in amyloplasts is different from that for L and M granules, as suggested previously (Bathgate and Palmer, 1972; McDonald et al., 1991).

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