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Physicochemical properties and structure of large, medium and small granule starches in fractions of normal barley endosperm

Hanjun Tang,[a] Hitomi Ando,[b] Katsumi Watanabe,[a] Yasuhito Takeda,[c] Toshio Mitsunaga[a,*]

[a]*Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Nara 631-0052, Japan*
[b]*Kyoto Bunkyo Junior Collage, Kyoto 611-0041, Japan*
[c]*Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan*

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Abstract

Normal barley grain was milled to flour with a machine used to polish brewers' rice from the surface layer to the center. Large (18.4 μm, median size), medium (12.3 μm) and small (2.2 μm) granule starches were isolated from classified flours. Their physicochemical properties and fine structure were investigated. The percentage (w%) of large granules decreased from the surface layer to the center, while the amounts of medium and small granules increased. Although all the starch granules were an A-type crystal, the relative crystallinity varied from 22.0 to 27.4%. The DP_n of the amyloses was around 1600 and similar for all the samples. But the amylose content of the starches varied from 21.9 to 26.4%. Also, the amylopectins showed differences in DP_n (around 5700–7900) and chain-length distribution between granule size or fractions. The transition temperature ranges and the enthalpy values of the starch granules differed with granule size. The gelatinization properties showed no correlation with any of the parameters, except the enthalpy value and relative crystallinity ($\gamma = +0.73$). The findings suggested that the structural characteristics of the starches in classified flours of normal barley differed essentially from those of waxy barley.

Keywords: Normal barley; Fraction; Starch; Property; Structure

1. Introduction

Barley is the world's fourth most important cereal after wheat, rice, and corn. It is the most widely cultivated, but for the most part, it is used for feed and brewing material rather than as a foodstuff for human consumption.[1] To promote the use of barley in food, we milled barley grain to flour with a machine used to polish brewers' rice.[1–3]

The major component of the classified flours is starch.[1,2] We have investigated starches from the classified flours of waxy barley (a six-rowed variety, *Hordeum vulgare* L.).[3,4] Large (12–19 μm median size), medium (10 μm) and small (2 μm) granule starches from the classified flours of waxy barley, dif-

Abbreviations: BV, blue value (starch–iodine determinations); CL, chain length; DP_n, degree of polymerization (number average); LC, longest chains; NC, number of chains/molecule.

* Corresponding author: Fax: +81-742-432252.
E-mail address: mitunaga@nara.kindai.ac.jp (T. Mitsunaga).

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fered in amount (w%) from the surface layer to the center of the grain. Also, the physicochemical properties and structural characteristics of the starches differed with granule size and among fractions. Takeda et al.,[5] who divided normal barley starch (a two-rowed variety, *Hordeum distichum* L.) into large, medium and small granules and characterized the structure of their amylose and amylopectin, indicated that the regulation or genetic control for the synthesis of the starches may be different. It is thought that the differences in characteristics with granule size of starch and with fraction of grain are important in final product applications of classified barley flours. This information also will help to elucidate the mechanism of biosynthesis of starch and to produce novel hybrids. This study aimed to investigate the physicochemical properties and structure of large, medium and small starch granules isolated from classified flours of normal barley.

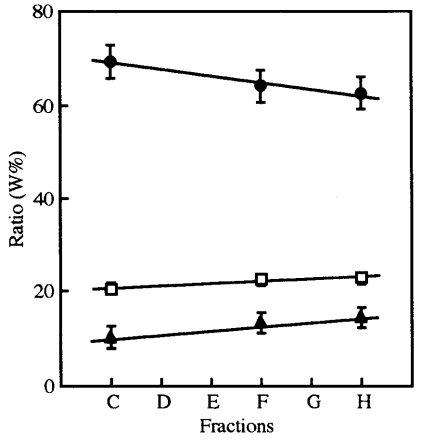

Fig. 1. Relative amounts (w%) of starch granules in the fractions of normal barley grains. The starches were fractionated into large (●), medium (□) and small granules (▲) by differential sedimentation

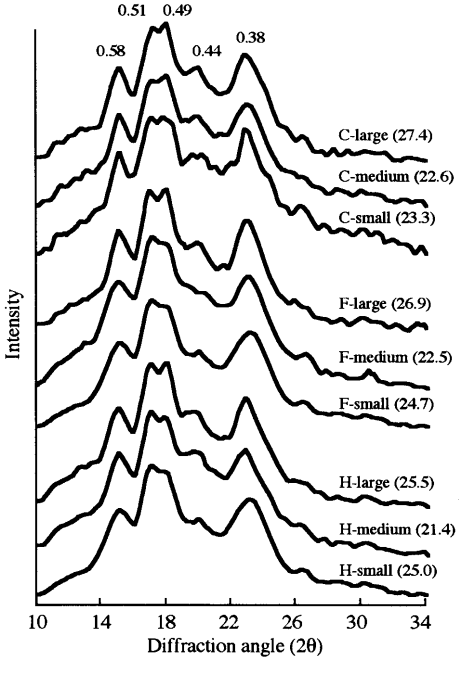

Fig. 2. X-ray diffraction patterns of starch granules from fractions of normal barley grains. Relative crystallinity (%); mean values of two separate measurements.

2. Results and discussion

Distribution of particle size.—The distribution of particle size for large, medium and small granules was around 7.7–44.9, 5.1–26.1 and 0.9–5.1 μm, respectively, with the median size being around 18.4, 12.3 and 2.2 μm, respectively, as determined with a particle size analyzer (Horiba, Ltd. LA-700 type). The relative amounts of large, medium and small granules is shown in Fig. 1, and is 69.2–62.5%, 20.6–23.0% and 10.2–14.5% (w%), respectively. The amount of large granules decreased from the surface layer of the grain to the center. On the other hand, the relative amounts of medium and small granules increased. The results were similar to those for waxy barley endosperm.[3]

X-ray diffractometry of starch granules.—The *X*-ray diffraction patterns of the starch granules are shown in Fig. 2. In all of the starches, major peaks were observed at a *d*-spacing of 0.58, 0.51, 0.49, 0.44 and 0.38 nm. Zobel reported that X-ray *d*-spacing of 0.58, 0.51 and 0.38 nm is characteristic of an A-type starch crystal that is common to most cereal starches.[6] The starches of waxy endosperm had the same characteristics.[3] The *d*-spacing of 0.44 nm is characteristic of an amylose–lipid complex. The peak was observed with large, medium and small granules in each fraction. The peak intensity changed little with granule size, but was higher than that of waxy endosperm as described previously.[3] The starch granules showed 22.0–27.4% relative crystallinity. The lowest value was for medium granules in the same fraction, while the largest

Table 1
Absorbance of the starch–iodine complex and amylose content of starches from the fractions of normal barley grain

Materials	λ_{max} (nm) [a]	BV [b]	Amylose content (%) [c]
C			
Large	629 ± 3.5	0.390 ± 0.003	25.9 (32.5)
Medium	627 ± 2.9	0.369 ± 0.012	24.6 (30.8)
Small	631 ± 2.4	0.348 ± 0.031	21.9 (29.0)
F			
Large	620 ± 6.8	0.383 ± 0.039	25.3 (31.9)
Medium	613 ± 1.5	0.377 ± 0.008	25.7 (31.4)
Small	610 ± 5.6	0.333 ± 0.001	22.0 (27.8)
H			
Large	628 ± 3.2	0.397 ± 0.022	26.4 (33.0)
Medium	628 ± 5.0	0.382 ± 0.055	25.4 (31.8)
Small	618 ± 4.7	0.328 ± 0.017	22.4 (27.3)

[a] Values are the mean $\pm$ SD of three separate measurements.

[b] Values are the mean $\pm$ SD of three separate measurements.

[c] Amylose content (%) = [BV(starch − amylopectin)/BV(amylose–amylopectin) $\times$ 100; ( ) apparent content (%) = [BV(starch)/BV(amylose) $\times$ 100, assuming the amylose BV to be 1.2.

Table 2
Properties of normal barley amylose molecules

Materials	λ_{max} (nm) [a]	BV [b]	DP_n [c]
C			
Large	665 ± 1.2	1.168 ± 0.037	1636 ± 51
Medium	663 ± 2.4	1.225 ± 0.010	1619 ± 123
Small	661 ± 0.3	1.231 ± 0.042	1679 ± 135
F			
Large	666 ± 2.5	1.210 ± 0.034	1647 ± 58
Medium	664 ± 1.5	1.226 ± 0.024	1617 ± 88
Small	656 ± 2.1	1.230 ± 0.011	1657 ± 152
H			
Large	665 ± 3.2	1.218 ± 0.024	1648 ± 120
Medium	666 ± 1.5	1.214 ± 0.041	1600 ± 57
Small	667 ± 1.2	1.232 ± 0.029	1636 ± 136

[a] λ_{max}, maximum absorption wavelength.

[b] BV, blue value at 680 nm. Values are the mean $\pm$ SD of three separate measurements.

[c] DP_n, number-average degrees of polymerization. Values are the mean $\pm$ SD of six separate measurements.

value was for large granules. Also, the values were low, compared to those of waxy barley endosperm.[3] Jenkins and Donald recently investigated the effect that varying amylose content has on the internal structure of maize, barley and pea starch species, and indicated that amylose disrupts the structure order within the amylopectin crystallites.[7] Thus, it was thought that the crystallinity of the normal endosperm starches was influenced by the amylose content.

Iodine absorption spectra and amylose content.—Absorbance of the starch–iodine complex and the amylose content of the normal barley starches are shown in Table 1. The λ_{max} of the starches was 610–631 nm. The BV for the small granules had the lowest value in the same fraction of the grain ($P < 0.01$). The apparent amylose content was higher (27.3–33.0%) than the corresponding true amylose content (21.9–26.4%), due to the BV of amylopectin.[5,8–11] The amylose content of small granules was less ($P < 0.01$) than that of the large and medium granules in the same fractions of the grain, but similar among the fractions. No correlations were found among amylose content, λ_{max} of the starches and relative crystallinity of starch granules. These results differed from those of waxy barley endosperm as described previously.[3,4] This suggested that, not only does the proportion of amylose–amylopectin in normal and waxy barley endosperm differ, but also the structural characteristics of the components. Thus, the amylose–amylopectin interaction in normal barley endosperm starches may be different.

Properties of the amylose molecule.—Properties of the amyloses from the large, medium and small granule starches of each fraction are shown in Table 2. The λ_{max}, BV and DP_n of the amyloses were 658–667 nm, about 1.2 and 1600, respectively. The values of λ_{max} were equal to those reported for waxy[4] and normal[5,11] barley amyloses. But the BV was lower than that of other normal barley amyloses reported previously.[5,11] The DP_n of the amyloses did not differ with granule size or among the fractions of grain. But, the values were about 400 glucose residues larger than those of waxy barley amyloses.[4] These values for the small granules also were about 300 glucose residues smaller than those of other normal barley amyloses,[5] probably due to a difference of cultivar. Although the DP_n of

amylose in all the plant starch examined differed, the λ_{max} showed a value that must be considered approximate,[4,5,11] probably due to a mixture in amyloses. To explain these results and to confirm that the amylose molecules are the same or different among granules or fractions, the distribution of molecular weight, proportion and characteristics of linear-branched molecules should be examined.

Structure of amylopectin.—Properties of the amylopectin molecule are shown in Table 3. The λ_{max} and BV of the amylopectins was 544–549 nm and 0.073–0.116, respectively. The BV of the large granules was significantly high ($P < 0.001$) compared to the values for medium and small granules in the same fraction. Also, the values were higher than those of waxy barley amylopectins[4] but similar to those of normal barley amylopectins reported previously.[5,11] The DP_n of the amylopectins varied from 5700 to 7900. The DP_n differed significantly between the large and small granules in the C fraction ($P < 0.05$), and between the C and H fractions ($P < 0.05$). This is the reverse of the result for waxy barley among its fractions.[4] The values also were smaller compared to waxy barley amylopectins.[4] The CL of the amylopectins was 19–20 residues. The values among the fractions were similar. The values of CL were greater than those of waxy amylopectins (17–19 residues),[4] and similar to those of normal barley amylopectins reported by others.[5,11] The NC of the amylopectins varied from 300 to 400, and differed between C, F and H fractions ($P < 0.01$). There were no significant correlations among the DP_n of the amylopectin and the relative crystallinity and enthalpy value of the starch granules. This was different from the results for waxy barley endosperm previously described.[4]

When the amylopectin, debranched by isoamylase, was fractionated on two columns, TSKG3000SW and TSKG2000SW (Fig. 3), one minor peak and three major peaks (RI detector response) were obtained. The profile of the major peaks was similar to that reported for waxy amylopectins.[4] All the amylopectins had LC (longest chains), similar to other normal barley amylopectins reported previously.[5,11] The major peaks were fractionated into three fractions, F.1–F.3, in a manner described previously.[4] The results are summarized in Table 4. The weight proportions of LC were 3.0–6.4%. The values differed among fractions C, F and H ($P < 0.005$). Takeda et al.[5] reported that the LC of normal barley was 2–3% (w/w), and small granules had a high value. Schulman et al.[11] indicated

Table 3
Properties of normal barley amylopectin molecules

Materials	λ_{max} (nm) [a]	BV [a]	DP_n [b]	CL [c]	NC [d]
C					
Large	549 ± 1.5	0.116 ± 0.002	7883 ± 369	20	393
Medium	548 ± 0.3	0.099 ± 0.001	7781 ± 840	19	409
Small	546 ± 1.2	0.097 ± 0.004	7050 ± 412	19	370
F					
Large	549 ± 1.5	0.112 ± 0.003	6881 ± 611	20	322
Medium	546 ± 2.9	0.085 ± 0.004	6423 ± 502	19	338
Small	546 ± 0.6	0.082 ± 0.004	6040 ± 408	20	301
H					
Large	549 ± 3.2	0.107 ± 0.002	6078 ± 588	20	303
Medium	546 ± 0.3	0.079 ± 0.005	6025 ± 504	20	294
Small	544 ± 1.2	0.073 ± 0.001	5726 ± 516	19	300

[a] λ_{max}, maximum absorption wavelength. Values are the mean ± SD of three separate measurements.
[b] DP_n, number average degrees of polymerization. Values are the mean ± SD of six separate measurements.
[c] CL, average-chain length. Values are the mean of six separate measurements.
[d] $NC = (DP_n/CL) - 1$.

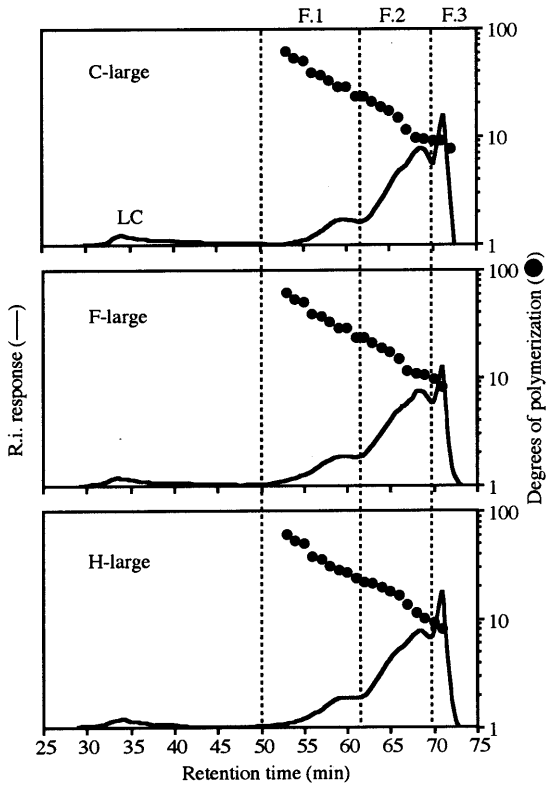

Fig. 3. Gel-permeation HPLC of isoamylase-debranched normal barley amylopectins. LC, longest chain. Columns, TSK gel G3000SW and G2000SW, 7.5 × 600 mm at 35 °C; buffer, 0.1 M NaOAc containing 0.02% NaN_3 (pH 6.2); flow rate, 0.6 mL/min.

that LC of normal and *shx* mutant of barley (*Hordeum vulgare* L.) were 4.3% and 4.8% (w/w), respectively. This difference is probably due to the cultivars, because those of normal rice[9] and normal maize[10] are different. The molar proportions of F.1 (9.2–10.7%) were similar among large, medium and small granules in the same fraction, but significantly different between fractions C and H ($P < 0.001$). The DP of F.1 showed similar values in all of the samples, except small granules of fraction C. Also, the values were similar to those of other barley[5,11] and rice.[8] The molar proportions of F.2 and F.3 were 50.0–59.3% and 31.4–39.6%, respectively, and similar among large, medium and small granules in the same fraction. But the values in F.2 and F.3 differed between fractions C and H ($P < 0.05$). The DP of F.2 and F.3 was 15.4–17.9 residues and 9.8–11.3 residues, respectively, and the values were also similar among the granules of the same fraction. But the values differed between fractions C and H ($P < 0.05$). The average DP of the short chain (F.2 and F.3) was 13–15 residues. The values were similar among the granules of the same fraction, but differed between fractions C and H

Table 4
Properties of chain-length distribution in normal barley amylopectins [a]

Samples	LC [b]	F.1 [c]			F.2			F.3			SF	SF/LF [d]	
		W%	M%	DP	W%	M%	DP	W%	M%	DP	DP	W	M
C													
Large	6.4	25.9	9.3	41.2	51.4	59.3	16.2	16.3	31.4	10.7	13	3.1	9.8
Medium	6.3	23.8	9.3	42.2	52.1	58.4	16.4	17.9	32.3	10.1	14	3.6	9.8
Small	6.0	24.0	9.2	47.4	50.8	57.6	15.4	19.2	33.2	10.9	13	3.8	9.9
F													
Large	4.5	24.6	9.4	41.8	51.6	56.7	16.7	19.4	33.9	10.1	14	3.2	9.6
Medium	4.0	24.0	9.2	42.5	51.0	54.4	15.9	21.0	36.4	10.1	14	4.0	9.9
Small	3.2	22.9	10.3	42.0	51.2	51.9	17.7	22.7	37.8	9.8	15	4.1	8.7
H													
Large	4.3	23.5	10.7	41.7	50.4	52.9	17.9	21.8	36.4	11.3	15	4.1	8.3
Medium	4.0	23.2	10.3	41.4	50.3	51.2	17.4	22.6	38.5	10.8	14	4.2	8.7
Small	3.0	22.7	10.4	41.5	50.2	50.0	17.7	24.1	39.6	11.3	15	3.7	8.6

[a] Values are the mean of two separate measurements.
[b] LC, longest chain.
[c] F., fraction; W, weight; M, molar; DP, average degrees of polymerization.
[d] SF, short chain fraction (F.2+F.3); LF, long chain fraction (F.1).

Table 5
Gelatinization properties of large, medium and small starch granules in normal barley grains

Samples	Gelatinization temperature (°C) [a]				Enthalpy (J/g) [b]
	T_o	T_p	T_f	$T_f - T_o$	
C					
Large	55.2	62.1	67.1	11.9	7.7 ± 0.1
Medium	52.0	62.2	68.2	15.2	5.7 ± 0.4
Small	53.3	64.5	69.0	15.7	5.0 ± 0.1
F					
Large	54.5	60.9	67.0	12.5	7.5 ± 0.5
Medium	53.9	61.2	68.1	14.2	5.5 ± 0.6
Small	54.4	63.6	69.7	15.3	5.3 ± 0.2
H					
Large	56.0	61.6	67.0	11.0	7.7 ± 0.7
Medium	52.2	62.6	69.1	16.9	5.8 ± 0.3
Small	52.2	64.0	69.7	17.5	5.4 ± 1.0

[a] Temperature (°C): T_o, onset; T_p, peak; T_f, final.
[b] Values are the mean $\pm$ SD of three separate measurements.

($P < 0.05$). The molar ratios of the short chain fraction to the long chain fraction (SF/LF) varied from 8.3 to 9.9. The ratios were similar among the granules of the same fraction, but significantly different between fractions C and H ($P < 0.001$). In addition, the linear correlations among parameters of the amylopectin structure were analyzed. The DP_n of the amylopectins showed high correlations with the molar proportions of F.2 ($DP_n = -5621.10 + 224.36$ F.2, $\gamma = +0.97$, $n = 9$) and F.3 ($DP_n = 15973 - 262.49$ F.3, $\gamma = -0.97$, $n = 9$), and with the molar ratio of SF/LF ($DP_n = -2110.70 + 946.98$ SF/LF, $\gamma = +0.79$, $n = 9$). The BV values of the amylopectins had positive correlations with the weight proportion of LC ($BV = 0.056 + 8.29$ LC, $\gamma = +0.70$, $n = 9$), and was similar to those of rice amylopectins.[9] Also, the BV of the amylopectins had positive and negative correlations with the molar proportions of F.2 ($F.2 = 38.39 + 172.85$, $\gamma = +0.78$, $n = 9$) and F.3 ($F.3 = 50.26 - 156.30$, $\gamma = -0.83$, $n = 9$), respectively. The intercept of the regression line gave a value of 0.056 at zero LC amylopectin. This was smaller than the average value of BV (around 0.066) of waxy barley amylopectins.[4] These results suggested that LC, F.2 and F.3 differ in their effect on the BV of amylopectin. Thus, it was indicated that LC, F.2 and F.3 are included in the side chains of the amylopectin molecule as reported.[9,12] The large amylopectin molecule also had more intermediate long chains and fewer short chains than the small molecule in normal barley starches. But the short chains were longer than those of waxy barley amylopectins.[4]

Gelatinization properties of starch granules.—Gelatinization properties of the starch granules from normal barley endosperm are listed in Table 5. The transition temperatures and enthalpy values of the starch granules were 52.0–69.7 °C, and 5.0–7.7 J/g, respectively. The values are smaller than those of the waxy barley endosperm starches.[3] The onset temperatures of large granules (around 55.0 °C, $P < 0.1$) are higher than those of the medium (52.0 °C) and small granules (53.0 °C). The peak (around 64.0 °C, $P < 0.05$) and final temperatures (69.5 °C, $P < 0.1$) of small granules are higher than those of the large and medium granules of the same fraction. The resulting transition temperature ranges (11.0–17.5 °C) show a tendency to increase with granule size in each fraction. The enthalpy values of the large granules (7.5–7.7 J/g) are about 2 J/g higher ($P < 0.01$) than those of the medium and small granules. The values are similar to those of other bar-

leys.[13–15] In addition, the enthalpy value has a positive correlation with the relative crystallinity (enthalpy $= -3.43 + 0.39$ relative crystallinity, $\gamma = +0.73$, $n = 9$), although the coefficient is lower than that of waxy barley endosperm.[3]

The crystallinity of the starch granule is associated with the amylopectin component. Cooke and Gidley[16] have indicated that the gelatinization of starch consisted of the process of the melting of the double helices and loss of crystallinity. Gelatinization represents the sum of individual crystal meltings.[17] Vasanthan and Bhatty maintain that the wide transition temperature range in the small granules of barley starches may be due to the high number of granules per unit weight of starch, compared to large granule starch.[15] Jenkins and Donald reported that reducing the amylopectin content (increasing the amylose content) has the effect of increasing the size of the crystalline region.[7] The enthalpy values in waxy barley endosperm show a decrease with granule size in the same fraction and increase from the surface layer to the center.[3] The enthalpy values also have a higher correlation with the relative crystallinity of the starch granules ($\gamma = +0.89$)[3] and the DPn of amylopectin ($\gamma = +0.80$),[4] respectively. In this study, the transition temperature range and enthalpy values of the starch granules differ significantly with granule size in the same fraction, but not between fractions unlike for waxy barley endsoperm.[3] The transition ranges are wider than those of waxy barley endosperm starches,[3] but there is no significant correlation with amylose content. Also, although the enthalpy value has a lower correlation ($\gamma = +0.73$) with the relative crystallinity, there is no significant correlation with the DP_n of amylopectin. Thus, the gelatinization properties of normal barley endosperm starches are suggested to be related to a variety of factors including size, crystalline organization and ultrastructure of starch granule. The factors reflect clearly the proportion and structural characteristics of amylose and amylopectin and interaction between the components.

3. Experimental

Materials.—Mature normal barley grain (Ichibanbosi, a six-rowed variety, *Hordeum vulgare* L.) grown in Okayama, Japan in 1996 was used. The grains were polished and milled as described previously.[1–3] 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. Three fractions of size class 80–70 (C-fraction), 50–40 (F-fraction) and 30–0 (H-fraction) were used for the experiments. Crystalline *Pseudomonas* isoamylase and Shodex Standard P-82 were the product of Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). All other chemicals were purchased from commercial suppliers.

Preparation of starch granules.—Starch granules were prepared by the alkali method and differential sedimentation from classified normal barley flours as described previously.[3]

Fractionation of amylose and amylopectin.—The starches were fractionated into amylose and amylopectin as reported previously,[18] and the purities of samples were assessed by gel filtration on TOYOPEARL HW-75F and iodine absorption spectroscopy.[19] Large, medium and small granules (10 g dry weight) produced 2.2–2.3 g, 2.1–2.3 g and 1.9–2.0 g of amylose and 6.8–7.0 g, 6.9–7.1 g and 7.0–7.3 g of amylopectin, respectively.

Analytical methods.—The distribution of particle size of the prepared starch was determined by a particle size analyzer (Horiba, Ltd. LA-700 type).[3] X-ray diffractometry and gelatinization properties of starch granules were determined as described previously.[3] Relative crystallinity (%) was calculated as the ratio of the areas of crystalline and amorphous regions of X-ray diffractograms by the method of Hermans.[20] Isoamylolysis of starch was carried out following the procedure of Hizukuri.[21] Iodine absorption spectra and amylose content of starches,[22] average degrees of polymerization (DP_n) and average chain-length (CL)[23] were determined following a procedure reported previously. The average number of chains per molecule (NC) was

$(DP_n/CL) - 1$. The chain length distribution of amylopectin after isoamylolysis was determined by gel-permeation HPLC on two sequentially linked columns (TSK gel G3000SW and G2000SW) at 35 °C.[4] Total carbohydrate was measured by the phenol–sulfuric acid method,[24] and the reducing value was determined by the Park–Johnson method.[23]

Statistical analysis.—Statistical analyses of all the data were performed using Microsoft Excel.

4. Conclusions

The present results indicate that the relative amount of large granules decrease from the surface layer to the center of normal barley grain, and the amount of medium and small granules increase at a similar level to those of waxy barley endosperm. The relative crystallinity, gelatinization properties, iodine absorption spectra and amylose content for the starches are similar among the fractions of grain, but differ with granule size as well as with the waxy barley endosperm. The structures of amylopectin molecules also differ with granule size and fraction of grain. The structural characteristics in normal barley endosperm starches are essentially different from those in waxy barley endosperm. These findings suggest that the proportion and structural characteristics of the components contribute to the difference in amylose–amylopectin interaction.

References

1. Mitsunaga, T.; Shimizu, M.; Inaba, K.; Yoshida, T.; Hayashi, S. *Bull. Inst. Compr. Agr. Sci. Kinki Univ.* **1994**, *27*, 55–62.
2. Ando, H.; Iizuka, H.; Shimizu, M.; Yoshida, T.; Inaba, K.; Mitsunaga, T. *J. Jpn. Soc. Nutr. Food Sci.* **1996**, *49*, 113–118.
3. Tang, H.; Ando, H.; Watanabe, K.; Takeda, Y.; Mitsunaga, T. *Cereal Chem.* **2000**, *77*, 27–31.
4. Tang, H.; Ando, H.; Watanabe, K.; Takeda, Y.; Mitsunaga, T. *Cereal Chem.* (in press).
5. Takeda, Y.; Takeda, C.; Mizukami, H.; Hanasuiro, I. *Carbohydr. Polym.* **1999**, *38*, 109–114.
6. Zobel, H. F. *Starch/Stärke* **1988**, *40*, 1–7.
7. Jenkins, P. J.; Donald, A. M. *Int. J. Biol. Macromol.* **1995**, *17*, 315–321.
8. Takeda, Y.; Hizukuri, S.; Juliano, B. O. *Carbohydr. Res.* **1987**, *168*, 79–88.
9. Hizukuri, S.; Takeda, Y.; Maruta, N.; Juliano, B. O. *Carbohydr. Res.* **1989**, *189*, 227–235.
10. Takeda, Y.; Shitaozono, T.; Hizukuri, S. *Starch/Stärke* **1988**, *40*, 51–54.
11. Schulman, A. H.; Tomooka, S.; Suzuki, A.; Myllarinen, P.; Hizukuri, S. *Carbohydr. Res.* **1995**, *275*, 361–369.
12. Takeda, Y. *Denpun Kagaku* **1993**, *40*, 67–71; *Chem. Abstr.* **1993**, *119*, 7417.
13. Kang, M. Y.; Sugimoto, Y.; Kato, I.; Sakamoto, S.; Fuwa, H. *Agric. Biol. Chem.* **1985**, *49*, 1291–1297.
14. Naka, M.; Sugimoto, Y.; Sakamoto, S.; Fuwa, H. *J. Nutr. Sci. Vitaminol.* **1985**, *31*, 423–429.
15. Vasanthan, T.; Bhatty, R. S. *Cereal Chem.* **1996**, *73*, 199–207.
16. Cooke, D.; Gidley, M. J. *Carbohydr. Res.* **1992**, *227*, 103–112.
17. Banks, W.; Greenwood, C. T. *Starch and Components*; Wiley: New York, 1975.
18. Takeda, Y.; Hizukuri, S.; Juliano, B. O. *Carbohydr. Res.* **1986**, *148*, 299–308.
19. Takeda, Y.; Shirasaka, K.; Hizukuri, S. *Carbohydr. Res.* **1984**, *132*, 83–92.
20. Nara, S.; Mori, A.; Komiya, T. *Starch/Stärke* **1978**, *30*, 111–114.
21. Hizukuri, S. *Carbohydr. Res.* **1985**, *141*, 295–306.
22. Takeda, C.; Takeda, Y.; Hizukuri, S. *Cereal Chem.* **1983**, *60*, 212–216.
23. Hizukuri, S.; Takeda, Y.; Yasuda, M.; Suzuki, A. *Carbohydr. Res.* **1981**, *94*, 205–213.
24. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.