Some Properties of Large and Small Granules of Waxy Barley (*Hordeum vulgare* L.) Endosperm Starch

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Summary Large and small starch granules were isolated and characterized from mature barley kernels with waxy endosperms. The large granules of any given waxy cultivar contained more amylose than the small granules of the same cultivar. It was also found that large granules contained a greater amount of long amylopectin B chains and had a lower fraction III: fraction II ratio, one of the structural characteristics of amylopectin, than the small granules in the same cultivar. Small granules showed a wider range of gelatinization and smaller heat of gelatinization by differential scanning calorimetry.

Key Words waxy barley starch, endosperm starch, large starch granules, small starch granules, amylose content, chain distribution of amylopectin, gelatinization characteristics, DSC

In a previous study (1), we prepared large and small starch granules from mature grains of 6 cultivars of barley, one with waxy and 5 with non-waxy endosperms, and which showed various differences in structural characteristics and properties between the large and the small granules. Namely, the large granules of any given non-waxy cultivar contained more amylose than the small granules of the same cultivar. Also, large granules contained more long amylopectin B chains and had a lower ratio of fraction III to fraction II of amylopectin (shorter α -1,4-chains/longer α -1,4-chains), one of the structural characteristics of amylopectin, than the small granules. Large granules had a lower conclusion temperature for gelatinization and larger heat of gelatinization than the small granules, as de-

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terminated by differential scanning calorimetry (DSC).

The present paper describes the structural characteristics determined by enzymatic and chromatographic methods and the gelatinization characteristics determined by DSC of small and large starch granules prepared from mature kernels of several waxy barley cultivars.

MATERIALS AND METHODS

1) *Barley seeds.* Kernel and endosperm types of the 9 cultivars of waxy barley and one cultivar of non-waxy barley which were used are described in Table 1. A single-letter abbreviation was given to the non-waxy cultivar and double- and triple-letter abbreviations were given to waxy cultivars. These abbreviations are used in Tables 2–5 and throughout the text hereafter. All of the cultivars except for NM were grown at the farm of the Plant Germ-plasm Institute, Kyoto University. Sample seeds of NM were kindly provided by Dr. Shinkichi Kubo, Managing Director, Kirin Brewery Co., Ltd., Tokyo, Japan.

Table 1. Kernel and endosperm types of barley sample seeds.

Cultivar		Line No.	Endosperm type	Kernel type
Name	Abbreviation			
Bozu	B	OUJ 180	nonwaxy	naked
Yatomi Mochi	YAM	OUJ 183	waxy	naked
Bozu Mochi	BM	OUJ 467	waxy	naked
Dango Mugi	DM	OUJ 483	waxy	naked
Waxy Oderbrucher	WO	OUJ 485	waxy	covered
Sumire Mochi	SM	OUJ 601	waxy	naked
Tokushima Mochimugi	TM	OUJ 783	waxy	naked
Yonezawa Mochi	YOM	OUJ 784	waxy	naked
Masan Hadakamugi	MH	OUJ 043	waxy	naked
Nijo Mochimugi	NM	—	waxy	covered

2) *Enzymes.* Crystalline *Pseudomonas* isoamylase [EC 3.2.1.68] was purchased from Hayashibara Biochemical Laboratories, Inc., Okayama, Japan. Sources of glucose oxidase and peroxidase have been reported previously (2).

3) *Preparation of barley starch and fractionation of large and small granules.* Starch granules were prepared from mature barley grains by Watson's method (3) and purified by Schoch's method (4). Large and small granules were fractionated by a series of decantations as described earlier (1).

4) *Other methods.* Measurement of starch granular size, determination of iodine absorption spectra, gel filtration of *Pseudomonas*-isoamylase-debranched materials and differential scanning calorimetry (DSC) have been described elsewhere (1).

Table 2. Average size of large (L) and small (S) starch granules of several waxy and one non-waxy barley cultivars.

Cultivar	Average size (μm)		a/b
	L (a)	S (b)	
B ^a	15.4	4.3	3.6
YAM	15.5	3.2	4.8
BM	15.7	—	—
DM	14.3	3.1	4.6
WO	13.7	2.7	5.1
SM	14.8	2.5	5.9
TOM	14.8	2.7	5.5
YM	14.5	—	—
MH	17.6	2.9	6.1
NM	14.0	3.3	4.2

^a For abbreviation, see Table 1.

RESULTS

1) Average size of large and small starch granules of several waxy barley cultivars

Table 2 shows that the average sizes of large and small granules of waxy barley starch were 14–16 μm , except for MH (17.6 μm), and 3–4 μm , respectively.

2) Absorption spectra of starch-iodine complexes

Some characteristics of the iodine absorption spectra of several waxy barley starches are summarized in Table 3. Large granules showed absorption maxima (λ_{max}) at 534–556 nm and small granules at 523–548 nm. The large granules of any given waxy cultivar, each of YAM, BM, DM, WO, SM, TM, YOM, MH and NM, gave a longer λ_{max} and a larger blue value than the small granules of the same cultivar.

3) Elution profiles of debranched waxy barley starches by gel filtration

Table 4 summarizes the distribution of glucan in each fraction for *Pseudomonas*-isoamylase-debranched large and small starch granules of non-waxy, B, and 4 waxy barley cultivars, YAM, WO, SM and NM. Because only low yields of small granules were obtained, only the data for large granules are shown for the other 5 waxy cultivars, BM, DM, TM, YOM and MH. The amounts of Fr. I, amylose (1, 5), ranged from 3 to 10% in the large granules and from 1 to 4% in the small granules of the waxy barley cultivars. The ratio of Fr. III to Fr. II, which represents one of the structural characteristics of amylopectin (5, 6), ranged from 3.3 to 3.8 for large granules of the waxy cultivars and from 3.6 to 4.4 for small granules.

Table 3. Wavelength ($\lambda_{\max}$) and optical density at maximum absorbance and blue value of starch-iodine complexes of large (L) and small (S) starch granules of several waxy and one non-waxy barley cultivar.

Cultivar	Starch granules	$\lambda_{\max}$ (nm)	Optical density at maximum absorbance	Blue value at 680 nm
B ^a	L	588	0.502	0.389
	S	582	0.398	0.292
YAM	L	537	0.301	0.122
	S	527	0.238	0.077
BM	L	556	0.343	0.193
	S	548	0.285	0.154
DM	L	534	0.284	0.102
	S	526	0.216	0.072
WO	L	546	0.310	0.146
	S	530	0.240	0.088
SM	L	540	0.315	0.130
	S	528	0.254	0.085
TM	L	536	0.297	0.109
	S	527	0.254	0.085
YOM	L	537	0.292	0.109
	S	523	0.245	0.075
MH	L	540	0.308	0.128
	S	528	0.225	0.077
NM	L	544	0.308	0.140
	S	530	0.229	0.103

^aFor abbreviation, see Table 1.

4) Pasting characteristics of waxy barley starches by DSC

Table 5 shows the gelatinization characteristics of several waxy barley starches. The small granules of any given cultivar, each of YAM, WO, SM and NM, had a peak temperature for gelatinization (T_p) similar to that of the large granules of the same cultivar, but had a wider range of gelatinization temperature (from onset (T_o) to conclusion (T_c) temperatures) and a smaller heat of gelatinization than the large granules of the same cultivar.

Table 4. Distribution of *Pseudomonas*-isoamylase-debranched materials of large (L) and small (S) starch granules of several barley cultivars.

Cultivar	Starch granules	Distribution of starch materials (%) ^a				Fr. III
		Fr. I	Int. Fr.	Fr. II	Fr.III	Fr.II
B ^b	L	30.2	2.9	14.6	52.3	3.6
	S	22.9	2.0	14.5	60.7	4.2
YAM	L	5.5	4.0	18.8	71.6	3.8
	S	1.5	3.0	17.9	77.7	4.4
WO	L	8.2	4.0	18.1	69.7	3.8
	S	2.8	2.9	18.4	75.9	4.1
SM	L	5.4	3.2	19.2	72.2	3.8
	S	1.1	1.9	18.8	78.2	4.2
NM	L	7.2	2.6	19.0	71.2	3.7
	S	3.9	2.6	20.5	73.0	3.6
BM	L	10.4	2.3	19.5	67.9	3.5
DM	L	3.0	3.0	21.2	72.8	3.4
TM	L	3.9	3.8	21.6	70.7	3.3
YOM	L	3.3	1.9	19.8	75.0	3.8
MH	L	4.4	3.3	20.0	72.3	3.6

^a Each fraction (Fr.) was divided according to the following range of $\lambda_{\max}$ values of iodine-carbohydrate complexes: Fr. I, $\lambda_{\max} \geq 620$ nm, Intermediate Fr., $620 \text{ nm} > \lambda_{\max} \geq 600$ nm, Fr. II, $600 \text{ nm} > \lambda_{\max} \geq 540$ nm and Fr. III, $540 \text{ nm} > \lambda_{\max}$.

^b For abbreviation, see Table 1.

DISCUSSION

Large and small starch granules were fractionated by decantation from the mature kernels of 9 waxy and one non-waxy barley cultivars with a known history. In general, small granules represent about 90% of the total granule number but only 10% of the weight of the starch (7). Arbitrarily, a large granule is defined as one which has a diameter greater than $10 \mu\text{m}$ (the average value being $14\text{--}18 \mu\text{m}$) while a small granule is one which has a diameter less than $10 \mu\text{m}$ (the average value being $3\text{--}4 \mu\text{m}$), according to Banks and Greenwood (8).

Endosperm starches of waxy barley with a known history consisted of 1–10%

Table 5. DSC characteristics of large (L) and small (S) starch granules of several barley cultivars.^a

Cultivar	Starch granules	T_o (°C)	T_p (°C)	T_c (°C)	Heat of gelatinization (cal/g)
B ^b	L	61	63	66	2.7
	S	59	63	68	1.9
YAM	L	62	65	70	3.1
	S	59	64	70	2.3
WO	L	59	63	68	2.9
	S	58	64	72	2.0
SM	L	60	64	70	3.3
	S	58	65	73	2.0
NM	L	58	62	65	2.8
	S	59	64	71	2.5

^a T_o , onset temperature; T_p , peak temperature; T_c , conclusion temperature for gelatinization.

^b For abbreviation, see Table 1.

amylose according to the enzyme-chromatographic method (Table 4). There are two possible mechanisms for the deposition of amylose in waxy barley (9). In one, the amylose arises from mutation at the amyloplast level. In the other, a direct xenia effect is exerted through cross-pollination in the field, although this possibility seems very unlikely under the experimental conditions adopted in the present study.

Various differences in structural characteristics and properties were observed between the small and large granules of waxy barley starches, as in the case of non-waxy starches (1). First, the large granules of any given waxy cultivar contained more amylose than the small granules of the same cultivar (Table 4), in agreement with previous findings (9, 10). Secondly, the large granules of any given cultivar had a slightly lower Fr. III:Fr. II ratio than the small granules of the same cultivar except in the case of NM (Table 4). Thirdly, the large granules of any given cultivar had a narrower range of gelatinization temperature and a greater heat of gelatinization as determined by DSC than the small granules of the same cultivar.

MacGregor and Morgan (10) recently investigated the fine structures of amylopectins isolated from large and small starch granules of both non-waxy and waxy barley using enzyme-chromatography. The results indicated that amylopectin from large non-waxy starch granules had a slightly higher proportion of long chains compared with the other amylopectins present. However, amylopectins from both large and small granules of waxy barley starch had similar proportions of long

chains. MacGregor and Morgan concluded that the difference was unlikely to be significant, because of the errors inherent in these determinations.

Bathgate and Palmer (7) reported that the small starch granules isolated from mature barley kernels (cultivar Maris Otter) were far more resistant to gelatinization than the large granules on the basis of polarising microscopy and the adsorption of Congo Red dye. Wider ranges of gelatinization and smaller heats of gelatinization of the small granules of waxy barley starches compared with those of large granules were shown by DSC (Table 5), as in the case of non-waxy barley starch (1).

We have therefore demonstrated differences in structural characteristics and properties between small and large starch granules of mature kernels for at least 4 cultivars of waxy barley with a known history as well as 5 cultivars of non-waxy barley with a known history (1).

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