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Structure and functionality of large, medium and small granule starches in normal and waxy barley endosperms

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Abstract

The structure and functionality of large, medium and small starch granules from normal and waxy barley were examined. The median size of large, medium and small granules was 18.1, 11.4 and 2.2 μm and 17.2, 9.2 and 2.0 μm in normal and waxy starch, respectively. The amylose content was 25.9% for normal small starch granules, about 4% lower than that of large and medium granules. For waxy starches, the amylose content ranged 2.9–3.3% and was similar among the granules. The DPn of amylopectins was 6100–8900, and similar between the varieties but differing among the granules. The CL of the amylopectins was 17–20 glucose residues, and in both starches, shorter in small granules than in large and medium granules. The relative crystallinity was 20.3–23.9% for normal starch granules, and 33.0–37.1% for waxy starch granules. The transition temperatures and enthalpy changes were 55.6–71.7°C and 7.4–8.5 J/g and 58.9–77.2°C and 10.1–12.1 J/g for the normal and waxy starch granules, respectively. The small granules displayed the greatest swelling power and susceptibility to enzymes, and the fastest retrogradation both in normal and waxy barley. The difference of functionality among granules of the same variety exceeded those between the same granule sizes of different varieties. © 2002 Published by Elsevier Science Ltd.

Keywords: Barley; Starch; Amylose; Amylopectin

1. Introduction

Barley is the world's fourth most important cereal after wheat, rice, and corn. Barley endosperm is mainly composed of starch, and has many genotypes, waxy, normal and high amylose varieties, similar to other cereals. The physicochemical properties and structural characteristics of these barley starches have been reported (Czuchajowska, Klamczynski, Paszczynska & Baik, 1998; Lauro, Forssell, Suortti, Hulleman & Poutanen, 1999; Morrison, Scott & Karakalas, 1986; Morrison, Tester, Snape, Law & Gidley, 1993; Song & Jane, 2000; Tester & Morrison, 1990, 1992; Yoshimoto, Tashiro, Takenouchi & Takeda, 2000; Zheng, Han & Bhatty, 1998). The high amylose starch granules have a higher transition temperature and lower peak viscosity, whereas the waxy starch granules show a higher endothermal enthalpy, peak viscosity (Song & Jane, 2000; Yoshimoto et al., 2000; Zheng et al., 1998) and swelling factor (Czuchajowska et al., 1998). The amylose content is 0–9.1% for waxy starches, 23.0–32.7% for normal starches and 33.4–48.7% for high amylose starches (Czuchajowska et al., 1998; Song & Jane, 2000; Tester & Morrison, 1992; Yoshimoto et al., 2000; Zheng et al., 1998). The amyloses from high amylose starch varieties have a larger number of chains per molecule than normal amylose, although molar fractions of the branched amylose are similar to those of normal amylose (Yoshimoto et al., 2000). The amylopectins of the three barley genotypes have similar structural characteristics and short branch chains (Song & Jane, 2000; Yoshimoto et al., 2000).

However, barley and wheat starches are distinct from other starches in the distribution of granular size. The bimodal distribution of large and small granules, and the properties and structure of the granules have been reported (Bathgate & Palmer, 1972; Kang, Sugimoto, Kato, Sakamoto & Fuwa, 1985; Lauro et al., 1999; MacGregor & Ballance, 1980; MacGregor & Morgan, 1984; Naka, Sugimoto, Sakamoto & Fuwa, 1985; Vasanthan & Bhatty, 1996). Takeda, Takeda, Mizukami and Hanasuiro (1999) fractionated normal barley starch (a two-rowed variety, *Hordeum distichum* L.) to large, medium and small granules, and characterized the structure of their amylose and amylopectin. Normal (a six-rowed variety, *Hordeum vulgare* L.) and waxy (a six-rowed variety, *Hordeum vulgare* L.) barley starches show the distribution curves of three peaks with a particle-size analyzer (Tang, Yoshida, Watanabe & Mitsunaga, 1998; Tang, Ando, Watanabe,

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Takeda & Mitsunaga, 2000). The large, medium and small granules of barley starches have been investigated for structural characteristics (Tang, Ando, Watanabe, Takeda & Mitsunaga, 2001a,b). However, the functional properties of the granules of barley starch have not been examined in detail.

In this study, we investigated the distribution of particle size, amylose content, structures of amylose and amylopectin, crystallinity, swelling, retrogradation, gelatinization and susceptibility to enzyme for large, medium and small granules from normal and waxy barley endosperms. The properties of the starches were compared with their structure.

2. Experimental

2.1. Materials

Mature normal barley grain (Amaki, a six-rowed variety, *Hordeum vulgare* L.) grown in Okayama, Japan in 1996 was used. Waxy barley grain (WB-97, a six-rowed variety, *Hordeum vulgare* L.) was provided by Prof Naofumi Morita, Osaka Prefecture University, Japan. Beta-amylase from barley was purchased from Sigma Chemical Co. (St. Louis, MO). Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochemical Laborataries, Inc. (Okayama, Japan). Other chemicals, all reagent grade, were used without purification.

2.2. Preparation of starch granules

The large, medium and small starch granules were isolated by the alkali method and differential sedimentation from barley flour milled with a modified machine used for polishing brewers' rice as described previously (Takeda et al., 1999; Tang et al., 1998, 2000).

2.3. Fractionation of amylose and amylopectin

Fractionation of amylose and amylopectin was carried out by the procedure of Takeda, Hizukuri and Juliano (1986).

2.4. Particle size distribution of starch granules

The particle size distribution of starch granules was examined by a particle-size analyzer (Horiba, LA-700) (Tang et al., 1998, 2000).

2.5. Properties of starch components

Iodine absorption spectra of starches, amylose content, number-average degrees of polymerization (DPn) and average chain length (CL) were determined following procedures reported previously (Hizukuri, Takeda, Yasuda & Suzuki, 1981; Takeda, Takeda & Hizukuri, 1983). The average number of chains per molecule (NC) was (DPn/CL) − 1. Isoamylolysis of amylose and amylopectin was carried out following the procedure of Hizukuri (1985).

2.6. Measurement of swelling power

Swelling power was evaluated by a modified version of the method by McCormick, Panazzo and Hong (1991). Starch (0.1 g) was weighed in glass tubes with coated screw caps to which 5 ml of a 0.1% $AgNO_3$ solution was added. The tubes were placed in a shaking water bath at 70°C for 10 min and then transferred into a boiling water bath. After gelatinizing perfectly, the tubes were cooled in cold water (20°C) for 5 min and centrifuged at 1700 × g for 4 min. The supernatant was removed carefully and swelling power was determined as sediment weight (g/g).

2.7. Turbidity measurement of dispersed starch

The turbidity of each starch was measured using a modified version of the method of Adkins and Greenwood (Klucinec & Thompson, 1999). The 0.5% starch dispersion in 20% (v/v of water) dimethyl sulfoxide was transferred into 1.5 ml glass cuvettes. The contents of the cuvettes were degassed at 20°C (for 40 min) before the measurement of absorbance. The cuvettes containing dispersion were immersed in an ice water bath for 5 min, and absorbance was measured again. The absorbance of the dispersion was measured every 5 min, for 15 min, while standing at 20°C.

2.8. Wide-angle X-ray diffraction of starch granules

X-ray diffraction was performed with an X-ray diffractometer, the Rint-2000 (Rigaku Denki, Tokyo), operating at 40 kV and 80 mA. Diffractograms were obtained from 4° 2θ to 40° 2θ with a scanning speed of 8°/min and scanning step of 0.02° 2θ. The degree of crystallinity for the starch granules was evaluated as the ratio of the areas of crystalline and amorphous regions of X-ray diffractograms with Hermans' method (Nara, Mori & Komiya, 1978).

2.9. Gelatinization properties of starch granules

Gelatinization properties of the prepared starch granules were measured by sensitive DSC-8240D (Rigaku Denki, Tokyo). The samples with a starch-to-water ratio of 5 mg to 15 μl were sealed hermetically into an aluminum pan of 30 μl. Distilled water was used as a reference material. The temperature was raised from room temperature (about 25°C) to 80°C at a rate of 5°C/min (Tang et al., 2000).

2.10. Susceptibility of starch granules to enzymes

To the sample (25 mg), 1 ml of 0.1 M acetate buffer (pH 4.8), 100 units of beta-amylase and 700 units of isoamylase, were added successively. The reaction was initiated at 37°C with shaking for 0–30 h. It was stopped by the addition of 50 μl of 1 M HCl and then the pH returned to 7.0 with a 1 M NaOH solution. The reaction mixture was pipetted into

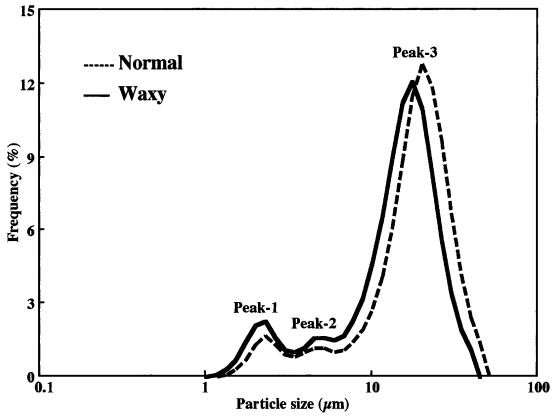

Fig. 1. Distribution of particle size of barley endosperm starches. Peak-1, -2 and -3: small, medium and large granules.

0.5 ml of 95% ethanol, and then centrifuged at 1500 × g for 10 min. The supernatant was analyzed for soluble carbohydrate by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). Percent hydrolysis was expressed as milligrams of maltose released per 100 mg of dry starch. Appropriate controls without the enzymes were prepared. The precipitate was washed with ethanol and diethyl ether, and dried in a vacuum dessicator. It was observed by SEM (Datam JSM-5400 LV, Japan) at an accelerating voltage of 15–30 kV (Tang et al., 1998).

3. Results and discussion

3.1. Particle size distribution of barley starches

In our earlier study, three peaks of particle size distribution were obtained in normal and waxy barley starches with a particle-size analyzer (Tang et al., 1998, 2000).In the present study, both normal and waxy barley starches showed three peaks of particle size distribution (Fig. 1). The range and proportion (vol.%) of small, medium and large granule peaks were 1.51–3.5 μm and 7.1%, 3.5–7.0 μm and 5.5% and 7.0–44.9 μm and 87.5% for normal starch, and 1.15–3.5 μm and 10.4%, 3.5–7.0 μm and 7.6% and 7.0–39.2 μm and 82.0% for waxy starch (Table 1). The normal starch had a wider distribution than waxy starch, but in both, the large granule comprised a major component by volume, while the medium and small granules comprised minor components. The weight proportions obtained by differential sedimentation were similar to these results (Table 1), and also agreed with the earlier reports (Takeda et al., 1999; Tang et al., 2000, 2001b). However, the distribution range of medium granules obtained with differential sedimentation was wider (5.1–26.1 μm) than that of medium granules both in normal and waxy starches, and the proportion was slightly higher. The median size of large, medium and small granules was 18.1, 11.4 and 2.2 μm in normal starch, and 17.2, 9.2 and 2.0 μm in waxy starch, respectively. The large and medium granules of normal starch were larger than those of waxy starch were. The results also differed slightly from earlier reports (Takeda et al., 1999; Tang et al., 2000, 2001b), probably due to different genotypes. However, the average diameter (2.0–2.3 μm) of small granule starches of all varieties investigated by differential sedimentation (Takeda et al., 1999; Tang et al., 2000, 2001b) was the same, although the proportions in different genotypes differed.

3.2. Properties of barley starches

The properties of starch, amylose and amylopectin of large, medium and small granule starches are shown in Table 2. The amylose content of the starches, calculated from the BV of starch, amylose and amylopectin, was 25.9% for normal small granules, being about 4% lower than that of large and medium granules but 5% higher than that of a two-rowed variety (Takeda et al., 1999) and similar to the value for a six-rowed variety (Tang et al., 2001b). The amylose content for waxy small granules was 2.9%, similar to that for waxy medium granules, but slightly lower than that for waxy large granules. The λ_{max} of waxy starches was 525 nm for large, medium and small granules. Their BV was 0.035–0.040. The λ_{max} and BV of normal amylopectins ranged 528–542 nm and 0.066–0.122, respectively, and decreased in the order of large, medium

Table 1
Distribution of particle size and proportion of large, medium and small starch granules (values are the mean (±SD) of three separate measurements)

Properties	Normal			Waxy		
	Large	Medium	Small	Large	Medium	Small
Unfractionation[a]						
Distribution (μm)	7.0–44.9	3.5–7.0	1.51–3.5	7.0–39.2	3.5–7.0	1.15–3.5
Ratio (vol.%)	87.5 (±2.4)	5.5 (±1.0)	7.1 (±1.4)	82.0 (±3.1)	7.6 (±1.5)	10.4 (±1.3)
Fractionation[b]						
Distribution (μm)	7.7–44.9	5.1–26.1	0.9–5.1	7.7–39.2	5.1–26.1	0.9–5.1
Ratio (wt%)	86.4 (±1.9)	7.0 (±1.3)	6.6 (±0.6)	80.5 (±1.3)	10.4 (±1.5)	9.1 (±0.5)
Median size (μm)	18.1 (±0.6)	11.4 (±0.5)	2.2 (±0.3)	17.2 (±0.2)	9.2 (±0.9)	2.0 (±0.1)

[a] Data from Fig. 1.
[b] Data obtained by the sedimentation method.

Table 2
Properties of barley starches (values are the mean of at least three separate measurements)

Properties	Normal			Waxy[a]		
	Large	Medium	Small	Large	Medium	Small
Starch						
λ_{max} (nm)[b]	622	627	626	525	525	525
BV[c]	0.456	0.452	0.369	0.040	0.035	0.035
Amylose (%)[d]	29.9	31.4	25.9	3.3	2.9	2.9
Amylopectin						
λ_{max} (nm)	542	538	528	525	525	522
BV	0.122	0.096	0.066	0.005	0.004	0.003
DPn[e]	8000	7400	6100	8900	7900	6200
CL[f]	20	19	19	19	18	17
NC[g]	400	389	321	468	438	365
Amylose						
λ_{max} (nm)	661	665	666			
BV	1.240	1.230	1.236			
DPn	1700	1700	1700			
CL	157	153	186			
NC	10	10	8			

[a] Amylose of waxy barley was not studied because of the limited amount of sample available.
[b] Maximum absorption wavelength.
[c] Blue value at 680 nm.
[d] Amylose content (%) of normal barley = [BV(starch − amylopectin)/BV(amylose − amylopectin)]100; amylose content (%) of waxy barley = (BV of starch/1.2]100.
[e] Number-average degrees of polymerization.
[f] Average chain-length (isoamylolysis).
[g] Average number of chains per molecule (= DPn/CL − 1).

and small granules. The results were similar to those of earlier reports (Schulman, Tomooka, Suzuki, Myllarinen & Hizukuri, 1995; Takeda et al., 1999; Tang et al., 2001b). The λ_{max} and BV of waxy amylopectins were the same for large and medium granules, but slightly lower for small granules. The λ_{max} of waxy large and medium granules was the same for amylopectins and starches, respectively. The BV of waxy amylopectins (0.003–0.005) was lower than that of normal amylopectins. The values were also lower than those of a waxy barley amylopectin reported previously (Tang et al., 2001a). The λ_{max} of waxy amylopectins agreed with a value of 524.4 nm extrapolated from the relationship between amylose content and λ_{max} of waxy barley starch at zero amylose content (Tang et al., 2000a).

The DPn of amylopectins ranged 6100–8900, and decreased in the order of large, medium and small granules in both normal and waxy barley. But the DPn of amylopectin was 500–900 glucose residues greater for waxy large and medium granules than normal large and medium granules, and similar between small granules (DPn 6100–6200). The DPn of the amylopectins was similar to that of *shx* mutant barley (Schulman et al., 1995), but smaller than for japonica rice (Takeda, Hizukuri & Juliano, 1987), and greater than that for indica rice (Takeda et al., 1987). The CL of the amylopectins was 17–20 glucose residues, and 1–2 residues longer in normal amylopectins than in waxy amylopectins. The CL for small granules was shorter than for large and medium granules in both normal and waxy barley. The values corresponded to those of earlier reports (Schulman et al., 1995; Takeda et al., 1999; Tang et al.,

Table 3
Swelling power (g/g) of barley starches (values are the mean ±SD of two seperate measurements, Nd = not determined)

Sample	Normal			Waxy		
	Large	Medium	Small	Large	Medium	Small
Granular starch	10.8 ± 0.1	12.2 ± 0.3	16.4 ± 0.8	13.8 ± 0.1	23.4 ± 0.7	25.7 ± 1.3
Amyloprotein[a]	13.8 ± 0.1	16.2 ± 0.6	21.0 ± 0.9	14.3 ± 0.1	24.1 ± 0.7	26.5 ± 1.3
Amylose	3.6 ± 0.2	3.4 ± 0.1	3.1 ± 0.1	Nd	Nd	Nd

[a] Swelling power of normal amylopectin = (swelling power of starch − swelling power of amylose × amylose content)/amylopectin content; swelling power of waxy amylopectin = swelling power of starch/amylopectin content.

Table 4
Turbidity (A650 nm) of 0.5% dispersions of barely starches in 20% (v/v) DMSO (values are the mean ± SD of two seperate measurements, Nd = not determined)

Treatment	Normal			Waxy		
	Large	Medium	Small	Large	Medium	Small
20°C (degassing)[a]						
Starch	0.060 ± 0.019	0.053 ± 0.004	0.083 ± 0.013	0.065 ± 0.004	0.078 ± 0.011	0.115 ± 0.002
Amylopectin	0.082 ± 0.032	0.081 ± 0.032	0.092 ± 0.025	0.066 ± 0.001	0.096 ± 0.004	0.163 ± 0.044
Amylose	0.004 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	Nd	Nd	Nd
Ice water (5 min)[b]						
Starch	0.446 ± 0.056	0.450 ± 0.054	0.492 ± 0.047	0.197 ± 0.018	0.191 ± 0.014	0.245 ± 0.023
Amylopectin	0.180 ± 0.034	0.230 ± 0.033	0.290 ± 0.080	0.190 ± 0.011	0.195 ± 0.009	0.260 ± 0.054
Amylose	1.837 ± 0.009	1.911 ± 0.097	1.928 ± 0.110	Nd	Nd	Nd
20°C (15 min)[c]						
Starch	0.074 ± 0.014	0.071 ± 0.013	0.101 ± 0.030	0.069 ± 0.007	0.081 ± 0.009	0.148 ± 0.041
Amylopectin	0.087 ± 0.031	0.090 ± 0.028	0.137 ± 0.063	0.071 ± 0.008	0.109 ± 0.006	0.174 ± 0.047
Amylose	Nd	Nd	Nd	Nd	Nd	Nd

[a] Turbidity after degassing at 20°C for 40 min.
[b] Turbidity after degassing at 20°C for 40 min and then standing in ice water for 5 min.
[c] Turbidity after standing in ice water for 5 min and then at 20°C for 15 min.

2001a,b). The amylopectin molecules contained 320–400 branch chains, and the waxy amylopectins another 40–70 chains. The amylopectins of small granules had an average of 60–100 chains less than the large and medium granules. The λ_{max}, BV and DPn of normal amyloses were 661–666 nm, 1.230–1.240 and 1700, respectively, and the same among large, medium and small granules. The values corresponded to those of earlier reports (Schulman et al., 1995; Takeda et al., 1999; Tang et al., 2001b). The amyloses of large and medium granules contained the same number of branch chains (NC, 10) but two chains more than those of small granules. The values were greater than those of a two-rowed barley (Takeda et al., 1999), but similar to those of an earlier report (Tang et al., 2001b). The amyloses of waxy starches were not examined because of the limited amounts of samples available. The results suggested that the amyloses and amylopectins resembled those of earlier reports (Tang et al., 2001a,b) in structural characteristics.

3.3. Swelling power of barley starches

The swelling power of barley starch components is given in Table 3. The swelling powers were 10.8–16.4 for normal starch granules, and 13.8–25.7 for waxy starch granules, and, increased in the order of large, medium and small granules. The normal amyloses had a swelling power of 3.1–3.6, similar among the granules. However, because of amylopectin gel, which was not separated from water by centrifugation, the swelling power of amylopectin could not be determined in this method. Therefore, the swelling power of amylopectins calculated on basis of amylopectin content following the method by Tester and Morrison (1992). The value was 13.8–21.0 for normal barley and 14.3–26.5 for waxy barley, and increased in the order of large, medium and small granules similar to the starches. The swelling power for the large granule amylopectins was similar among normal and waxy barley, but higher for the waxy medium and small granule amylopectins than for normal medium and small granule amylopectins, respectively. These observations were similar to those of waxy and normal barley starches reported previously (Vasanthan & Bhatty, 1996). Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinization and replaced by hydrogen bonds with water (Tester & Karkalas, 1996). The swelling power of starch depends on the capacity of starch molecules to hold water via hydrogen bonding (Lee & Osman, 1991). In barley and wheat, starch amylose content was reported to correlate with lipid content, and lipid-complexed amylose reduced the swelling power of starch (Tester & Morrison, 1990, 1992, 1993). These studies examined the swelling of starch at lower temperatures. Sasaki and Matsuki (1998) investigated the swelling power of wheat starches at the boiling point, and indicated that amylose content correlated negatively with the swelling power of starch, but starch lipid content showed no such correlation. In the present, the large granule amylopectins with the highest BV and the longest CL had the lowest swelling power. In addition, the waxy amylopectins with a lower BV and shorter CL had greater swelling power than normal amylopectins. Amylopectins with a short average branch chain length contained a large proportion of short branch chains (Jane, Chen, Lee, McPherson, Wong, Radosavljevic et al., 1999). Waxy barley had a higher molar ratio of short chains to long chains in amylopectins than normal barley, but in both varieties, large granule amylopectins contained a lower molar proportion of A-chains than medium and small granules (Tang et al., 2001a,b). The amylopectins were similar in properties and structures to

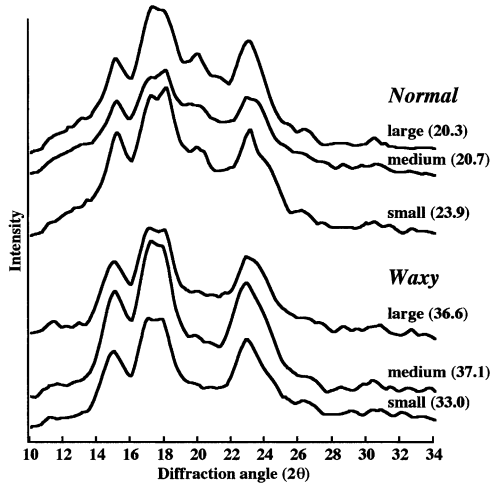

Fig. 2. X-ray diffraction patterns of large, medium and small granule starches. Relative crystallinity (%).

the amylopectins presented here. Thus, when starch molecules after gelatinizing completely are replaced by hydrogen bonds with water, amylose content and the proportion of outside-chains of amylopectin may be the major factors stabilizing the gel structure to retain water.

3.4. Turbidity of barley starches

To observe the retrogradation behavior of barley starches and starch fractions, The turbidity of starch dispersions in DMSO–water solvent was determined at different temperatures (Table 4). The value for the amylose dispersions was similar among large, medium and small granules in each observation. The turbidity was near zero after degassing for 40 min at room temperature (20°C). After cooling of the amylose dispersions in the ice-water bath for 5 min, samples became highly turbid, and the turbidities reached 1.837–1.928. However, because of a clear upper layer that formed in the cuvette, above the turbid region during holding at room temperature after removal from the ice-water bath, the exact turbidity could not be determined in this step. The observations during cooling and rewarming agreed with an earlier report (Klucinec & Thompson, 1999). The turbidity of the normal amylopectin dispersions was highest after degassing and rewarming for 15 min at room temperature and lowest after cooling in the ice-water bath, while the turbidity of the normal starch dispersions was intermediate between that of the amyloses and amylopectins. In addition, the starch and amylopectin dispersions of normal small granules had higher turbidities than those of normal large and medium granules. For waxy starches and amylopectins, the observations were similar to those for normal amylopectins. In the steps of degassing and rewarming for 15 min, the amylopectins had higher values than the starches, probably due to its solubility in solvent. The results suggested that the small granule starches were the easiest to retrograde both in normal and waxy barley. The differences among granules may be due to the different molar concentrations, because the DPn of amylopectin among the large, medium and small granules differed greatly.

3.5. X-ray diffractometry of starch granules

The X-ray diffraction patterns of the starch granules are shown in Fig. 2. In all the starches, major peaks were observed at a *d*-spacing of 0.58, 0.51, 0.49, 0.44 and 0.38 nm. Zobel (1988) has reported that a *d*-spacing of 0.58, 0.51 and 0.38 nm is characteristic of an A-type crystal that is common to most cereal starches; the *d*-spacing of 0.44 nm is characteristic of amylose–lipid complex. The peak was observed clearly at 0.44 nm with normal large, medium and small granules, and reflected a difference of amylose content from waxy starch granules. The relative crystallinity of normal starch granules was 20.3–23.9%, a little higher for small granules than large and medium granules. The values were lower than those of normal barley reported earlier (Tang et al., 2001b). The relative crystallinity of waxy starch granules was 33.0–37.1%, a little lower for small granules than large and medium granules. The values were similar to or higher than those of waxy barley reported previously (Tang et al., 2000).

3.6. Gelatinization properties of starch granules

It is well known that the gelatinization of many natural starch granules involves two endotherms with DSC. The first endotherm depends on the crystallinity of starch granule, and the second endotherm is related to the amylose–lipid complex (Czuchajowska et al., 1998). The first endotherm was measured in the present study. The

Table 5
Gelatinization properties of large, medium and small granule starches (values are the mean (±SD) of two separate measurements)

Properties	Normal			Waxy		
	Large	Medium	Small	Large	Medium	Small
Temperature (°C)						
Onset (T_o)	57.0	55.6	58.4	59.7	58.9	60.5
Peak (T_p)	62.2	61.5	64.7	64.3	64.4	67.6
Final (T_f)	69.1	68.6	71.7	70.5	71.6	77.2
ΔT ($T_f - T_o$)	12.1 (±0.3)	13.0 (±0.2)	13.3 (±0.4)	10.8 (±0.3)	12.7 (±0.3)	16.7 (±0.4)
Enthalpy (ΔH, J/g)	8.5 (±0.5)	8.0 (±0.4)	7.4 (±0.6)	12.1 (±0.3)	11.2 (±0.5)	10.1 (±0.6)

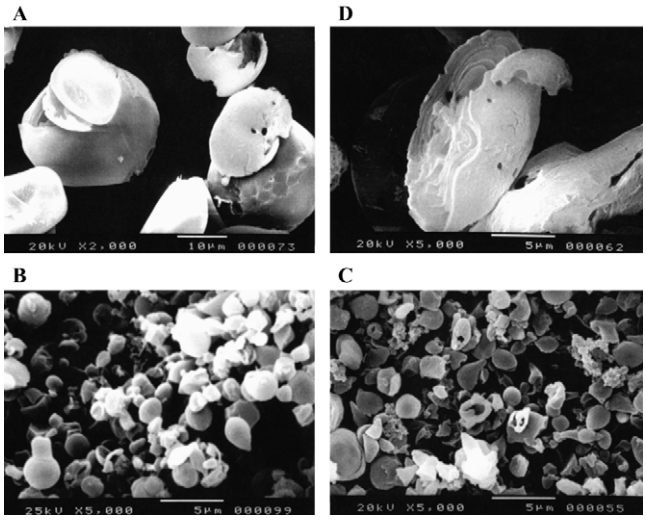

Fig. 3. SEM of barley starch granules hydrolyzed by beta-amylase and isoamylase. (A, B) normal large (for 5 h) and small (for 22 h) granules, (C, D) waxy large and small granules (for 30 h), respectively.

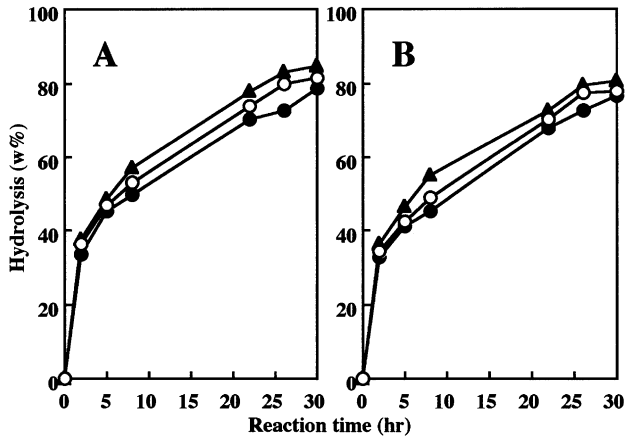

Fig. 4. Enzyme hydrolysis of barley starch granules. (A) normal; (B) waxy; (●) large granules; (○) medium granules; (▲) small granules.

transition temperatures and enthalpy changes are given in Table 5. The transition temperatures were 55.6–71.7°C for the normal starch granules, and 58.9–77.2°C for the waxy starch granules. The waxy starches had transition temperatures that 2–3°C higher than normal, and in both normal and waxy starches, small granules had a higher transition temperature than large and medium granules. The results were similar to those of earlier reports (Tang et al., 2000, 2001b). The transition temperature ranges (ΔT) were 12.1–13.3°C for the normal starches, and 10.8–16.7°C for the waxy starches. The enthalpy changes (ΔH) were 7.4–8.5 J/g for the normal starches, and 10.1–12.1 J/g for the waxy starches. For both normal and waxy starches, enthalpy changes were lower and transition temperature ranges wider in small granules and corresponded with those reported earlier (Kang et al., 1985; Naka et al., 1985; Tang et al., 2000, 2001b; Vasanthan & Bhatty, 1996). The higher gelatinization temperature range of small granules of barley starch may be due to the higher number of granules per unit weight of starch when compared to large granule; starch gelatinization represents the sum of individual crystal meltings (Vasanthan & Bhatty, 1996). Waxy starches are known to display larger gelatinization enthalpy changes, reflecting a greater crystallinity of amylopectin, which corresponded with our results. Larger amounts of energy were needed to gelatinize crystallites of longer chains; the relatively high ratio of short chains suggests a defective crystalline structure (Jane et al., 1999). The different enthalpy changes among the granules may be due to the branch chain lengths of amylopectin.

3.7. Susceptibility of starch granule to enzymes

The appearance of the starch granules hydrolyzed by conjugation of beta-amylase and isoamylase was studied by SEM (Fig. 3). All starch granules had numerous deep holes on their inside and a core hydrolyzed by the enzymes. The granules appeared to be composed of small, more or less spherical blocklets coming together tangentially. However, the blocklet was clearly greater in large granules than in small granules. These observations were consistent with those reported earlier (Tang et al., 1998). All the starch granules showed a relatively fast rate of hydrolysis initially (from 0 to 2 h), followed by a slower rate up until 30 h (Fig. 4). But the degree of hydrolysis increased ($P < 0.005$) in the order of large, medium and small granules in both normal and waxy barley. Also, the normal starch was hydrolyzed faster ($P < 0.005$) than the waxy starch. The different rates before and after hydrolysis corresponded with those of acid and alpha-amylase hydrolysis (Vasanthan & Bhatty, 1996). This is interpreted as showing that the enzymes attack the more amorphous regions of the starch granules initially; whereas the less accessible crystalline regions are hydrolyzed at a slower rate. The differences between normal and waxy starches may be due to amylose content and crystallinity. However, the differences in the extent of hydrolysis were greater among different granules of the same variety than between same sized granules of different varieties. These results suggested that the crystalline size or the stability of the crystalline structure differed greatly among the different sized granules of the same variety.

4. Conclusions

Differences in components, crystallinity, gelatinization properties and retrogradation behavior were greater among the genotypes than among granules of barley starch, whereas the reverse was true of swelling power and susceptibility to enzymes. The differences in DPn, swelling power and retrogradation behavior of barley amylopectins were smaller among the genotypes than among the granules.

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