

# Molecular structure and some physicochemical properties of waxy and low-amylase barley starches

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Received 14 September 2000; accepted 18 December 2000

## Abstract

The molecular structures and physicochemical properties of starches from an actual waxy (HB 340) and two low-amylase (CDC Candle and Sumiremochi) cultivars of barley were examined and compared with those of a normal cultivar (CDC Dawn). HB 340 starch had no detectable amylose. The amylose content was 2.2 and 11.4% in CDC Candle and Sumiremochi starches, respectively, being much lower than that in CDC Dawn starch (25.4%). The starches of HB 340, CDC Candle and Sumiremochi showed a higher viscosity and breakdown on the viscomgram than CDC Dawn starch, and HB 340 starch showed the highest  $\Delta H$  of peak 1 and no peak 2 on the DSC thermogram. The amylopectins resembled each other in average chain-length (19–20),  $\beta$ -amylolysis limit ( $\beta$ -AL 53–54%) and chain-length distribution. The amyloses of CDC Candle and Sumiremochi were larger (DPn 1680 and 1560, respectively) and contained the branched amylose with a lower number of chains (NC 6.1–10.4) than CDC Dawn amylose (DPn 1220, NC 13.8). CDC Candle amylose had a higher molar fraction (45%) of the branched molecule than the others (21–26%). © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Waxy barley; Low-amylase barley; Starch; Amylose; Amylopectin

## 1. Introduction

Barley is one of the major cereals along with rice, maize and wheat, and has many cultivars, which have starches with different amylose content. We recently examined two high-amylase barley starches on molecular structures of fractionated amylose and amylopectin in detail together with some physicochemical properties (Yoshimoto, Tashiro, Takenouchi & Takeda, 2000). The high-amylase barley starches were of the A crystalline type like that of the normal barley starch, but showed no or very low viscosity on a rapid viscomgram. Their amyloses were similar in size and contained a similar amount (by mole) of the branched amylose to the normal barley amylose, but their branched amyloses were highly branched. Their amylopectins were similar in the molecular structure to that from the normal

cultivar, such as average chain-length,  $\beta$ -amylolysis limit and chain-length distribution.

Some molecular structures of waxy and low-amylase barley starches having an amylose content of 1.7–9.1% were investigated (Czuchajowska, Klamczynski, Paszczynska & Baik, 1998; Fredriksson, Silverio, Andersson, Eliasson & Aman, 1998; MacGregor & Morgan, 1984; Song & Jane, 2000; Tester & Morrison, 1992). The results indicated that their amylopectins were similar in structure. However, their amyloses have not been examined as to the molecular structure.

Bhatty and Rossnagel (1997) recently produced an actual waxy barley cultivar, which was entirely free of amylose, by breeding two types of waxy barley cultivars. Zheng, Han and Bhatty (1998) examined their physicochemical properties. However, their molecular structures have not been examined. It is of interest how molecular structures are different or similar among actual waxy, low-amylase, normal and high-amylase cultivars. Here, we examined the molecular structures of amylose and amylopectin from waxy and low-amylase barley cultivars, together with some physicochemical properties of their starches, and compared them with those of normal and high-amylase barley cultivars.

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Table 1

Iodine affinity and amylose content of barley starches

Property	Waxy	Low-amylose		Normal
	HB 340	CDC Candle	Sumiremochi	CDC Dawn
Iodine affinity, IA (g/100 g)				
Defatted starch (A)	0.00	0.97	2.38	5.49
Starch (B)	0.00	0.36	1.10	3.92
A – B	0.00	0.61	1.28	1.57
Amylose content (%) <sup>a</sup>	0.0	2.2	11.4	25.4
Apparent amylose content (%) <sup>b</sup>	0.0	4.9	11.9	27.5

<sup>a</sup> Calculated by  $(IA_{\text{defatted starch}} - IA_{\text{amylopectin}})/(IA_{\text{amylose}} - IA_{\text{amylopectin}}) \times 100$ . IA values of amylopectins and amyloses are in Tables 4 and 6, respectively.<sup>b</sup> Calculated by  $(IA_{\text{defatted starch}}/20) \times 100$ .

## 2. Materials and methods

### 2.1. Materials

Barley grains of four cultivars, HB 340, CDC Candle, Sumiremochi and CDC Dawn (normal cultivar), were used in this study. HB 340, CDC Candle and CDC Dawn were gifts from Dr. Rossnagel, Crop Development Center, University of Saskatchewan (SK, Canada), and Sumiremochi was obtained from Kagoshima Prefectural Agricultural Experiment Station (Kagoshima, Japan). Starch was isolated from barley grains by an alkaline steeping method as reported previously (Takeda, Takeda, Mizukami & Hanashiro, 1999). Defatted starch was prepared by three replications of dissolution in dimethyl sulfoxide and precipitation with ethanol (Takeda, Hizukuri & Juliano, 1986). Amylose and amylopectin were fractionated by the method of Takeda et al. (1986). The purity of amylose specimens was confirmed by gel-permeation chromatography on Toyopearl HW-75F (Tosoh, Tokyo, Japan) (Takeda, Shirasaka & Hizukuri, 1984).  $\beta$ -Limit dextrin ( $\beta$ -LD) from amylose was prepared as reported (Takeda, Hizukuri, Takeda & Suzuki, 1987a). Crystalline *Pseudomonas* isoamylase was the product of Hayashibara Biochemical Lab. Inc., (Okayama, Japan). Sweet potato  $\beta$ -amylase (Sigma Chemical Co., St. Louis, MO) was further purified by the method of Marshall and Whelan (1973).

### 2.2. Physicochemical analyses

Iodine affinity (IA, g/100 g) was determined by the amperometric titration method (Larson, Gilles & Jenness, 1953) with modification (Takeda, Hizukuri & Juliano, 1987b). Pasting properties of starch slurry at a concentration of 9% (w/w) in the presence and absence of 3-methyl-1-butanol were determined by a Rapid Visco Analyzer with a paddle rotated at a fixed 160 rpm (RVA-3D, Newport Scientific, Narrabeen, Australia). The starch slurry was heated from 40 to 92.5°C at a rate of 3.0°C/min, maintained at 92.5°C for 15 min, and then cooled to 40°C at the same rate. Thermal behavior of starch was determined by differ-

ential scanning calorimetry (DSC-7, Perkin Elmer, Norwalk, CT) as described previously (Yoshimoto et al., 2000). X-ray diffraction was performed on an X-ray diffractometer (Rotaflex RV-20013, Rigaku Denki Co., Tokyo, Japan) under the conditions described by Hizukuri, Takeda, Shitaozono, Abe, Ohtakara, Takeda et al. (1988).

### 2.3. Analytical methods

The blue value,  $\lambda_{\text{max}}$  and  $\beta$ -amylolysis limit ( $\beta$ -AL) were determined as described (Suzuki, Hizukuri & Takeda, 1981; Takeda, Takeda & Hizukuri, 1983). The number-average degree of polymerization (DPn) was determined by a modification of the Park–Johnson method (Hizukuri, Takeda, Yasuda & Suzuki, 1981; Takeda et al., 1987b). The average chain-length (CL) was determined by the rapid Smith degradation (Hizukuri & Osaki, 1978; Hizukuri et al., 1981) and hydrolysis with isoamylase (Suzuki et al., 1981). The average number of chains per molecule (NC) was calculated as DPn/CL. Carbohydrates and reducing sugars were determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and Somogyi (1952)–Nelson (1944) method with a minor modification (Hizukuri, Tabata & Nikuni, 1970), respectively. Phosphorus was determined as inorganic phosphate (Fiske & Subbarow, 1925) after treatment with hot perchloric acid (Allen, 1940). Phosphorus linked to C-6 of the glucosyl residue was assayed as glucose-6-phosphate using glucose-6-phosphate dehydrogenase (Hizukuri et al., 1970). The chain-length distribution of amylopectin debranched with isoamylase was examined by gel-permeation HPLC with a refractive index (RI) and low-angle laser light-scattering photometer as detectors (Hizukuri, 1986) and by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Hanashiro, Abe & Hizukuri, 1996). DPn and molar-based distribution of amylose were determined using fluorescent-labeled amylose as a sample by gel-permeation HPLC with fluorescence and RI detectors (Hanashiro & Takeda, 1998).

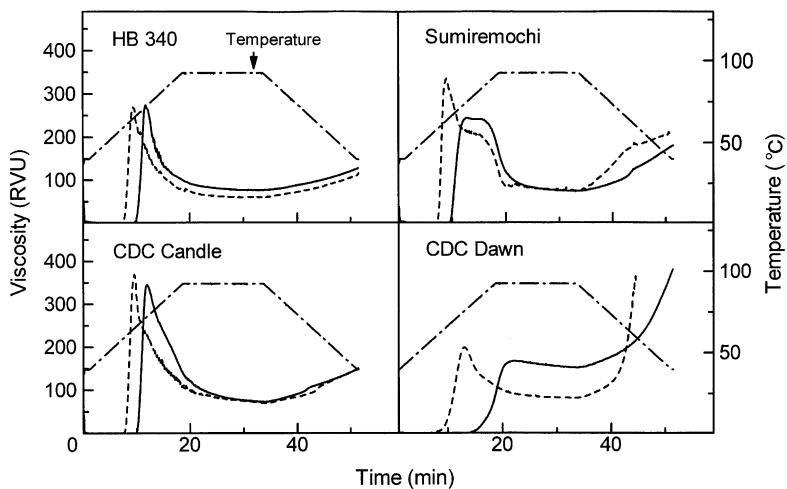


Fig. 1. RVA viscograms of barley starches. —, pasting with water; - - - and aqueous 5% 3-methyl-1-butanol.

### 3. Results and discussion

#### 3.1. Amylose content

Table 1 summarizes the iodine affinity (IA, g/100 g) and amylose content of barley starches. HB 340 starch showed an undetectable IA value, implying that the starch was an actual waxy-cultivar as reported by Bhatty and Rossnagel (1997). The difference between IA before and after defatting of CDC Candle starch was smaller than the values for Sumiremochi and CDC Dawn, indicating that CDC Candle starch had a smaller amount of amylose-lipid complex than the others. The true amylose content, calculated from IA values of defatted starch, amylose and amylopectin, was 2.2% for CDC Candle and 11.4% for Sumiremochi, being much lower than that for CDC Dawn (25.4%).

Thus, CDC Candle had a very small amount of amylose. Therefore, we here regarded CDC Candle as a low-amylase cultivar as well as Sumiremochi, although CDC Candle was registered as a waxy cultivar (Zheng et al., 1998). The true content was similar in the case of Sumiremochi to the apparent amylose content, which was calculated without consideration of amylopectin IA, but was 2–3% lower for CDC Candle and CDC Dawn. This slight discrepancy was caused by a slightly high IA of their amylopectins as described later. The difference in these amylose contents was similar to those of other normal and high-amylase barley starches (0–4%) (Takeda et al., 1999; Yoshimoto et al., 2000), but considerably lower than those of indica rice (7–11%) (Takeda et al., 1987b) and amylomaize starches (9–18%) (Takeda, Takeda & Hizukuri, 1989) because their amylopectin IA was relatively high.

Table 2  
Pasting properties of barley starches (conc. 9%)

Property	HB 340	CDC Candle	Sumiremochi	CDC Dawn
<b>Water</b>				
Pasting temperature (°C)	64.6	65.7	64.6	77.9
Maximum viscosity, $V_{\max}$ (RVU)	276	347	219	164
Temperature at $V_{\max}$ (°C)	72.2	72.6	74.4	92.5
Time at $V_{\max}$ (min)	11.8	12.0	12.6	21.9
Minimum viscosity, $V_{\min}$ (RVU)	76	75	66	152
Viscosity at 40°C, $V_{40^{\circ}\text{C}}$ (RVU)	126	149	161	371
Breakdown, $V_{\max} - V_{\min}$ (RVU)	209	272	153	16
Set back, $V_{40^{\circ}\text{C}} - V_{\min}$ (RVU)	53	74	95	219
<b>5% 3-Methyl-1-butanol</b>				
Pasting temperature (°C)	58.3	58.8	56.8	58.3
$V_{\max}$ (RVU)	271	370	302	198
$V_{\min}$ (RVU)	59	70	69	90
$V_{40^{\circ}\text{C}}$ (RVU)	113	151	189	— <sup>a</sup>
Breakdown (RVU)	212	300	233	90
Set back (RVU)	54	81	120	— <sup>a</sup>

<sup>a</sup> Not determinable.

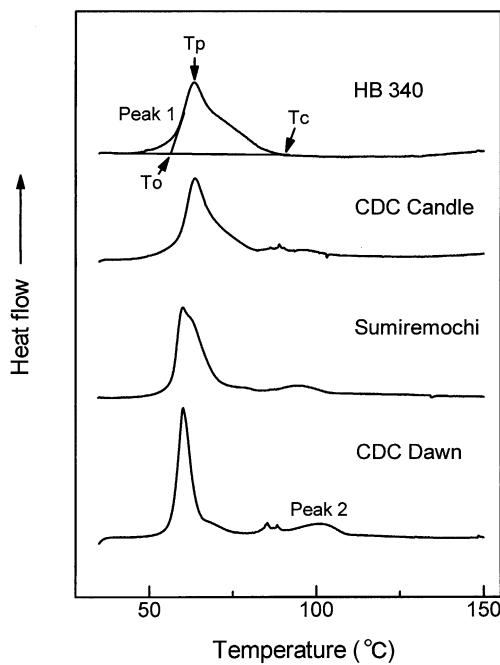


Fig. 2. Thermograms of differential scanning calorimetry (DSC) for barley starches.  $T_o$ ,  $T_p$ , and  $T_c$  are onset, peak, and complete transition temperatures, respectively.

### 3.2. Pasting properties

Fig. 1 shows viscograms of barley starches (9%, w/w) determined by a rapid viscoanalyzer, and Table 2 the pasting properties. HB 340, CDC Candle and Sumiremochi starches showed a lower pasting temperature (64.6–65.7°C) but a higher maximum viscosity (219–347 RVU), and reached the maximum viscosity at a lower temperature (72.1–74.4°C) with a shorter period (11.8–12.6 min) than CDC Dawn starch. The minimum viscosity of HB 340, CDC Candle and Sumiremochi (66–76 RVU) was lower than that of CDC Dawn (152 RVU). The breakdown of starch from HB 340 (209 RVU), CDC Candle (272 RVU) and Sumiremochi (153 RVU) was more than 10 times larger than that

from CDC Dawn (16 RVU). The viscosity at 40°C and set back seemed to increase with the increase of amylose content. These results suggested that the waxy and low-amylose starches had greater swelling and more fragile properties and lower retrogradation tendency than the normal starch. Zheng et al. (1998) reported that the Brabender amylogram of CDC Candle starch showed a slow increase in viscosity at the early stage of pasting followed by a sharp increase, and the starch had a lower maximum viscosity and reached the maximum viscosity at a much higher temperature than starch from zero amylose HB, a similar waxy cultivar as HB 340. However, our CDC Candle starch showed no such slow increase in viscosity, and had a higher maximum viscosity and a similar temperature of the maximum viscosity when compared with HB 340 starch. The reason for these different behavior of pasting between two CDC Candle starches is unknown.

The pasting temperature of barley starches was lowered (6–20°C) by 5% 3-methyl-1-butanol (3-MB) but the maximum viscosity was increased except for HB 340. This behavior was similar to those of other cereal starches (Hizukuri & Takeda, 1978; Jideani, Takeda & Hizukuri, 1996; Takeda, Suzuki & Hizukuri, 1988), but differed from those of lily, potato and sago starches (Takeda et al., 1983; Takeda, Takeda, Suzuki & Hizukuri, 1989), since 3-MB raised the pasting temperature and decreased the maximum viscosity of these starches. The minimum viscosity, viscosity at 40°C, breakdown and set back were less affected by 3-MB for the waxy and low-amylose starches than for the normal starch. Sumiremochi starch showed a different swelling behavior from the others in both the presence and absence of 3-MB. The behavior suggested that the starch was composed of two kinds of granules differing in swelling properties. However, they were not distinguishable under a microscope even after iodine staining.

### 3.3. Differential scanning calorimetry

Fig. 2 shows differential scanning calorimetry (DSC) thermograms of barley starches and Table 3 their characteristic

Table 3  
Thermal properties of barley starches

Cultivar	Peak 1				Peak 2			
	$T_o^a$ (°C)	$T_p^b$ (°C)	$T_c^c$ (°C)	$\Delta H^d$ (J/g)	$T_o^a$ (°C)	$T_p^b$ (°C)	$T_c^c$ (°C)	$\Delta H^d$ (J/g)
HB 340	57.1	63.1	88.0	17.7	— <sup>e</sup>	— <sup>e</sup>	— <sup>e</sup>	— <sup>e</sup>
CDC Candle	58.5	63.6	81.3	15.0	88.3	95.8	104.4	0.3
Sumiremochi	56.9	60.9	76.7	14.0	86.3	94.9	103.6	0.7
CDC Dawn	56.8	60.0	75.0	12.9	85.4	102.7	108.9	3.3

<sup>a</sup> Onset temperature.

<sup>b</sup> Peak temperature.

<sup>c</sup> Complete temperature.

<sup>d</sup> Enthalpy change.

<sup>e</sup> Not detectable.

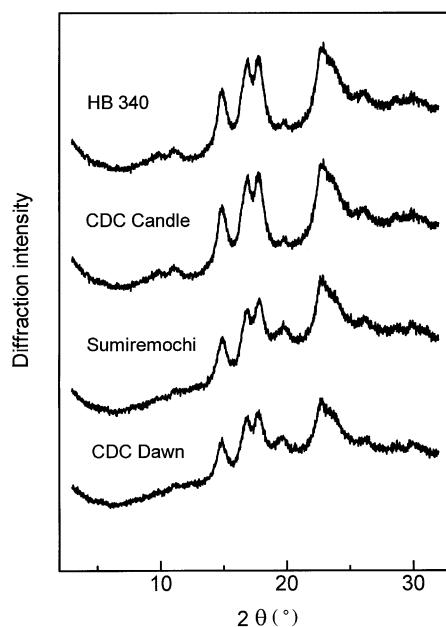


Fig. 3. X-ray diffractograms of barley starches.

values. HB 340 starch gave only one transition (peak 1) and had the highest  $\Delta H$  among them. The  $\Delta H$  values of CDC Candle and Sumiremochi were higher than that of CDC Dawn. These findings were probably due to a higher amylopectin content. The range of transition temperature ( $T_c - T_o$ ) was in the order of HB 340 > CDC Candle > Sumiremochi > CDC Dawn, the lower-amyllose starch showing a narrower range. Similar results have been reported for some waxy and low-amyllose barley starches (Gudmundsson & Eliasson, 1992; Morrison, Tester, Snape, Law & Gidley, 1993; Zheng et al., 1998). Thus the waxy and low-amyllose starches had a higher amount of crystallites than the normal starch because the first transition corresponding to melting of crystallites of starch, mainly amylopectin (Morrison et al., 1993). Sumiremochi starch had a shoulder peak near the main peak, showing behavior similar to the viscogram. The second transition (peak 2),

which corresponded to melting of the amylose–lipid complex (Kugimiya, Donovan & Wong, 1980), was not found for HB 340, but was found for CDC Candle, Sumiremochi and CDC Dawn starches. CDC Candle and Sumiremochi starch had a much lower  $\Delta H$  than CDC Dawn starch, indicating that the low-amyllose starches had a lower amount of amylose–lipid complex, being in agreement with the IA difference before and after defatting (Table 1).

### 3.4. X-ray diffraction pattern

HB 340 and CDC Candle starches belonged to the A crystalline type, as reported by Zheng et al. (1998), and Sumiremochi and CDC Dawn starches also showed the A crystalline type (Fig. 3), being the same type as high-amyllose barley starches (Czuchajowska et al., 1998; Song & Jane, 2000; Vasanthan & Bhatty, 1996; Yoshimoto et al., 2000). Thus, barley starches were of the same crystalline type regardless of amylose contents, suggesting that the amylopectins from these barley cultivars were similar in CL. In the case of maize, waxy and normal amylopectins were largely different in CL from amylomaize amylopectin, and the former starches were of the A type whereas amylomaize starch the B type (Hizukuri, Kaneko & Takeda, 1983).

### 3.5. Structure of amylopectin

The properties of the barley amylopectins are summarized in Table 4. HB 340 starch contained actually no amylose component, but its amylopectin was prepared from starch by the same procedure as for the other amylopectins. HB 340 showed no affinity for iodine. Sumiremochi amylopectin showed a poor affinity (IA 0.12) compared with CDC Candle (IA 0.55) and CDC Dawn (IA 0.65) amylopectins. A similar tendency was observed in the blue value and  $\lambda_{\max}$ . The amylopectins from all the cultivars were similar in CL (19–20) and  $\beta$ -AL (53–54%), but appeared to be different in molecular size, that is, the amylopectins in HB 340, CDC Candle and Sumiremochi (DPn 5700–8700) were

Table 4  
Properties of barley amylopectins

Property	HB 340	CDC Candle	Sumiremochi	CDC Dawn
IA (g/100 g)	0.00	0.55	0.12	0.65
Blue value	0.05	0.12	0.04	0.12
$\lambda_{\max}$ (nm)	522	540	537	548
Average chain length, CL				
Smith degradation	20	20	19	20
Isoamylolysis	19	20	20	20
$\beta$ -Amylolysis limit, $\beta$ -AL (%)	54	53	54	53
Number-average DP, DPn	7300	8700	5700	12000
Phosphorus				
Organic (ppm)	24	69	19	14
Linked to C-6 (ppm)	9	10	13	10

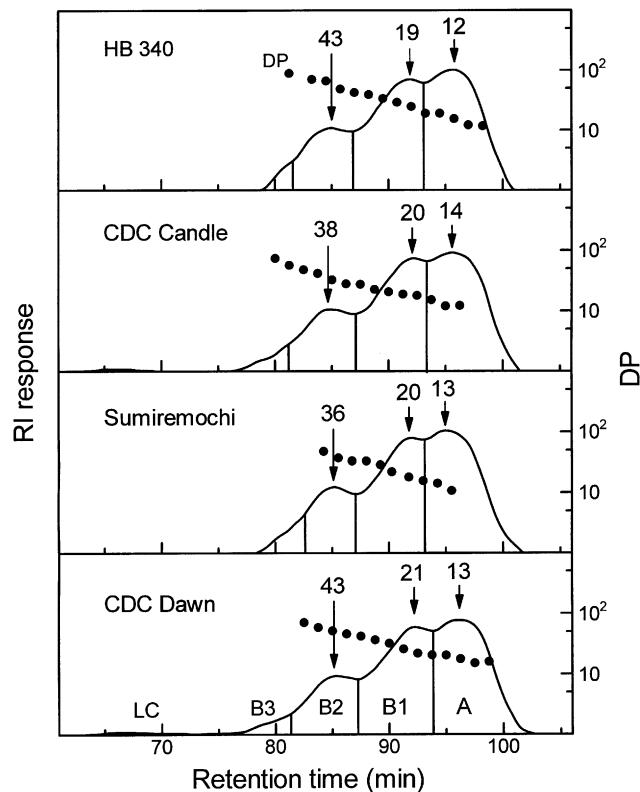


Fig. 4. Gel-permeation HPLC chromatograms of barley amylopectins after isoamylolysis. —, differential refractometry (RI); ●, DP.

smaller than those in CDC Dawn amylopectin (DPn 12,000), although CDC Dawn had a larger amylopectin than the other normal barley cultivars (DPn 6000–8700) (Schulman, Tomooka, Suzuki, Myllarinen & Hizukuri, 1995; Yoshimoto et al., 2000). The amylopectins had a small amount of phosphorus (14–69 ppm), and among them, CDC Candle amylopectin had the highest amount. A portion of phosphorus (about 10 ppm) was linked to C-6, and the remainder was supposed to bind to C-3, as in the case of potato amylopectin (Hizukuri et al., 1970). Thus, the amylopectins of waxy and low-amylase barley cultivars seemed to be similar in structure to those of other normal and high-amylase barley cultivars (Schulman et al., 1995; Takeda et al., 1999; Yoshimoto et al., 2000).

The chain-length distribution of the barley amylopectins was examined by gel-permeation HPLC after debranching

with isoamylase (Fig. 4). The chains were fractionated into five fractions, LC, B3–B1 and A, in the order of elution (Hizukuri, 1986). All the amylopectins were similar in the chain-length distribution and the carbohydrate amount of each fraction although the  $CL_w$  value for each fraction was somewhat different (Table 5). These chain-length distributions were also similar to those of high-amylase barley amylopectins (Yoshimoto et al., 2000). The LC fraction was reported to be absent in a waxy culvar of rice (Hizukuri, 1986) but to be present in normal rice cultivars in various amounts (7–20%) (Takeda et al., 1987b). HB 340 and Sumiremochi showed no LC fraction as in waxy rice, whereas CDC Candle and CDC Dawn had a very small but detectable amount ( $\leq 1\%$ ) of LC fraction (see also Fig. 4), as well as high-amylase barley cultivars (1–2%). The amount of LC fraction appeared to be related with amylopectin IA as reported previously (Takeda et al., 1987b). HPAEC-PAD analyses revealed a more detailed distribution of short chains below DP 60 (Fig. 5A). All the amylopectins had a peak of DP 11 or 12 and a shoulder of DP 18. Comparison of each peak area (Fig. 5B) indicated that all the amylopectins had the same chain-length distribution, but slightly differed from those of the high-amylase barley cultivars (Fig. 5C).

Both these and previous (Yoshimoto et al., 2000) results indicated that amylopectins from barley cultivars having a different amylase content were similar in molecular structure, confirming the previous findings (Czuchajowska et al., 1998; MacGregor & Morgan, 1984; Song and Jane, 2000; Tester and Morrison, 1992) in a more detailed extent. Similarities in the amylopectin structure appeared to be characteristic for barley cultivars, because significant differences were observed in IA, CL and chain-length distribution in maize and rice cultivars.

### 3.6. Structure of amylose

CDC Candle starch contained a very small amount of amylose (2.2%), but we isolated the amylose. All the amylose specimens were confirmed to be free from amylopectin by gel-permeation chromatography on Toyopearl HW-75F (Takeda et al., 1984). Table 6 summarizes the properties of amyloses. CDC Candle and Sumiremochi amyloses were a larger molecule (DPn 1680 and 1560, respectively) than CDC Dawn amylose (1220), although they were similar in IA (19.3–19.8), blue value (1.33–1.42)

Table 5

Carbohydrate amounts and weight-average chain length ( $CL_w$ ) of the fractions of isoamylase-debranched amylopectins

Cultivar	Amount (% of total)					$CL_w$				
	LC	B3	B2	B1	A	B3	B2	B1	A	$\sum(B3 - A)$
HB 340	0	3	18	37	42	89	50	25	15	27
CDC Candle	0	4	19	36	41	117	40	20	14	25
Sumiremochi	0	5	17	36	42	98	45	23	14	27
CDC Dawn	1	3	19	37	40	149	42	25	16	28

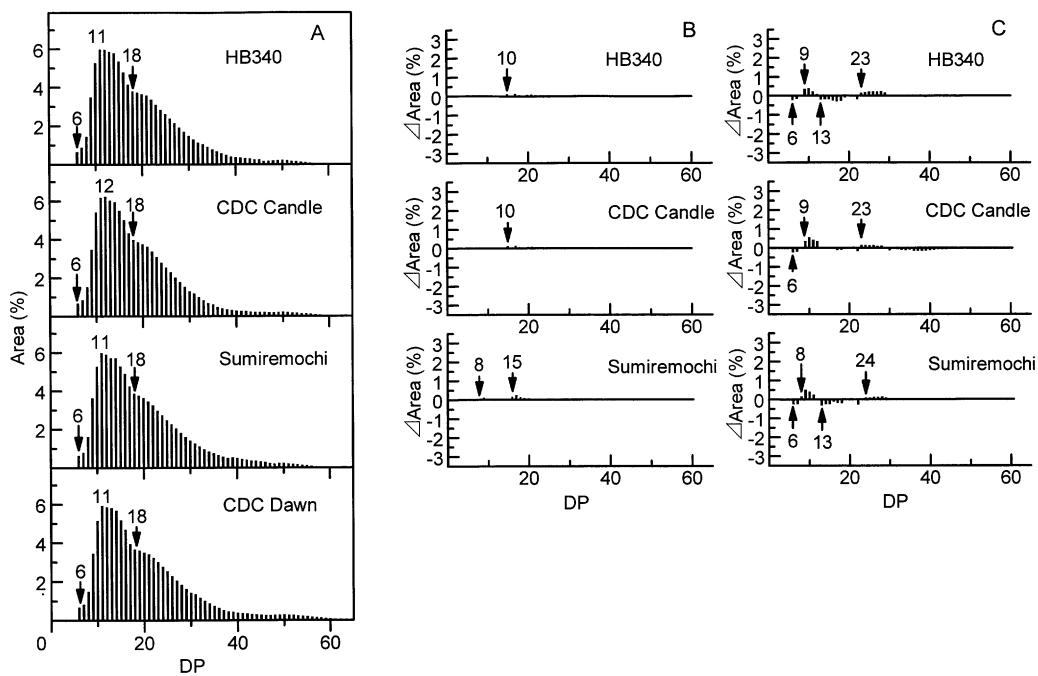


Fig. 5. Chain-length distributions of barley amylopectins (A) and differences in chain-length distribution against the CDC Dawn (B) and high-amyllose Glacier A (C).

and  $\lambda_{\max}$  (639–655 nm). The DPn values determined by the colorimetric method were close to those determined by the fluorescent labeling method. The DPn values of the low-amylose cultivars were higher than those (DPn 950–1080) of the high-amylose cultivars. The amyloses from the low-amylose cultivars had a slightly higher average-number of chains per molecule (3.3–3.4) than that (2.4–2.7) from the high-amylose cultivars. The  $\beta$ -amylolysis limit was 77–82%.

The fluorescent labeled amylose was examined by gel-permeation HPLC with RI and fluorescence detectors (Fig. 6). The RI and fluorescent profiles showing weight- and molar-based distributions, respectively, indicated that

CDC Candle and Sumiremochi amyloses had a peak and a shoulder similar to CDC Dawn amylose. The molar-based distribution showed that all the amyloses comprised two molecular species differing in molecular size, and the small species was predominant although the proportion slightly varied with the cultivar. These distributions were similar to those of amyloses from normal rice, wheat and maize (Hanashiro & Takeda, 1998).

Amylose comprised linear and branched amyloses. Since there are no methods available for the separation of these amyloses, we examined the structure of the  $\beta$ -limit dextrin ( $\beta$ -LD) derived from the branched amylose (Table 6). The

Table 6  
Properties of barley amyloses and their  $\beta$ -limit dextrans ( $\beta$ -LD)

Property	CDC Candle		Sumiremochi		CDC Dawn	
	Amylose	$\beta$ -LD	Amylose	$\beta$ -LD	Amylose	$\beta$ -LD
IA (g/100 g)	19.8	17.1	19.5	17.1	19.3	17.2
Blue value	1.35	1.30	1.42	1.29	1.33	1.23
$\lambda_{\max}$ (nm)	643	638	655	641	639	634
Dpn						
Colorimetric method <sup>a</sup>	1680	1840	1560	1450	1220	1440
Labeling method	1830	1930	1600	1330	1330	1250
CL	510	304	460	140	330	104
$\beta$ -AL (%)	82		77		79	
Average number of chains, NC	3.3	6.1	3.4	10.4	3.7	13.8
Molar fraction (%) of						
Branched amylose	45		26		21	
Linear amylose	55		74		79	

<sup>a</sup> Modified Park–Johnson method.

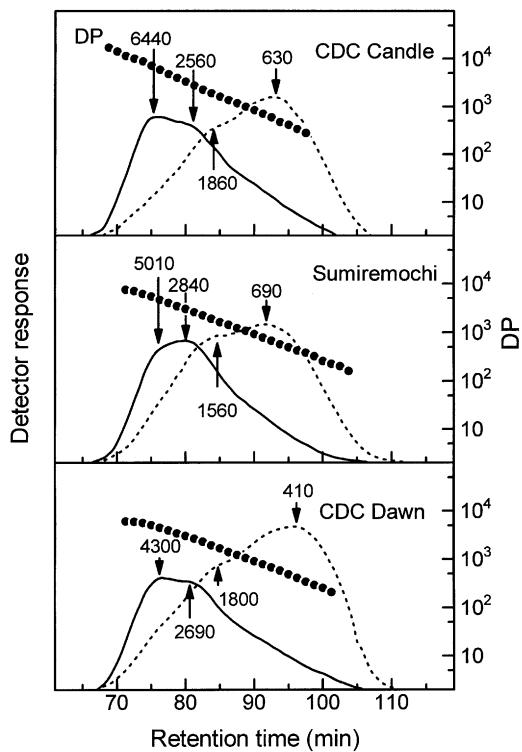


Fig. 6. Gel-permeation HPLC chromatograms of fluorescent-labeled amyloses. —, differential refractometry; ---, fluorescense response; ●, DP. Arrows indicate DP values.

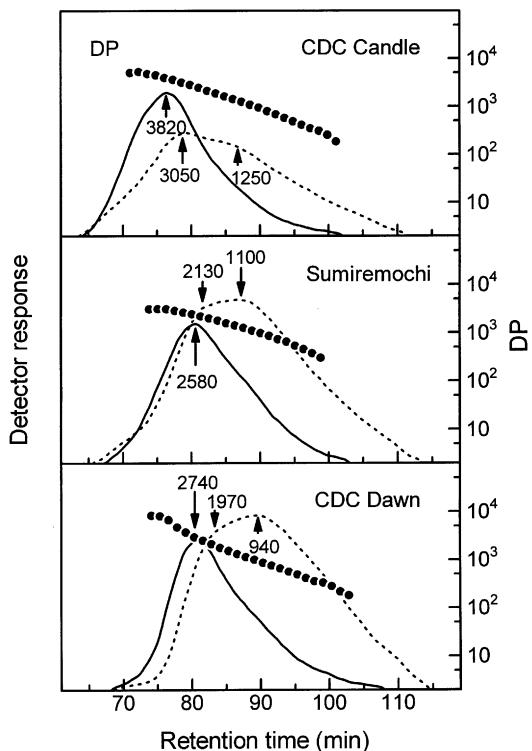


Fig. 7. Gel-permeation HPLC chromatograms of fluorescent labeled  $\beta$ -LDs. —, differential refractometry; ---, fluorescense response; ●, DP. Arrows indicate DP values.

$\beta$ -LDs of CDC Candle and Sumiremochi showed similar IA (17.1), blue value (1.29–1.30) and  $\lambda_{\text{max}}$  (638–641 nm) to that of CDC Dawn. The  $\beta$ -LD of CDC Candle was a larger molecule (DPn 1840) than those of Sumiremochi (DPn 1450) and CDC Dawn (DPn 1440), suggesting that CDC Candle had a larger branched amylose than the others. These DPn values were similar to those determined by the fluorescent labeling method. The CL values of  $\beta$ -LDs suggested that the branched amylose of CDC Candle had the longest inner chain among them. The branched amylose of CDC Candle, Sumiremochi and CDC Dawn had 6.1, 10.4 and 13.8 chains on average, respectively, because  $\beta$ -LD was derived from the branched amylose. These results indicated that the branched amylose of a lower-amylose cultivar were less branched, but no such tendency was observed when compared with other normal (5.8) and high-amylose (9.5–10.6) cultivars. CDC Candle amylose had the highest proportion of the branched amylose (45% by mole). Sumiremochi amylose had a low proportion (26%), similar to CDC Dawn (21%) and high-amylose cultivars (15–20%) (Yoshimoto et al., 2000).

The weight- and molar-based distributions of  $\beta$ -LDs are shown in Fig. 7. The weight-based distribution showed a single peak, differing from the parent amylose. The peak-top DP (3820) of CDC Candle was larger than those of Sumiremochi (2580) and CDC Dawn (2740), supporting that CDC Candle had a larger branched amylose than the others, as mentioned above. The molar-based distribution indicated that all the  $\beta$ -LDs were comprised of two species differing in molecular size, like their parent amylose, indicating that there were two species of the branched amylose in all the cultivars. The large species of the branched amylose was predominant in CDC Candle whereas minor in the other cultivars. The peak-top and shoulder DP values of  $\beta$ -LDs were larger than those of the respective parent amyloses. This finding suggested that the branched amylose was larger than the linear amylose on average.

In conclusion, the waxy and low-amylose barley starches showed a different physicochemical property from normal and high-amylose barley starches, such as a higher maximum viscosity and breakdown on the RVA viscomgram. The molecular structure of the amylopectins was similar in the waxy, low-amylose, normal and high-amylose barley cultivars. The similarities appeared to be characteristic for barley cultivars, which differed from the maize and rice cultivars. The amyloses from low-amylose cultivars were larger molecules than those from normal and high-amylose cultivars.

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