

Molecular Structure and Some Physicochemical Properties of High-Amylose Barley Starches

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ABSTRACT

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The molecular structure and some physicochemical properties of starches from two high-amylose cultivars of barley, high-amylose Glacier A (HAG-A) and N (HAG-N), were examined and compared with those of a normal cultivar, Normal Glacier (NG). The true amylose contents of HAG-A, HAG-N, and NG were 41.0, 33.4, and 23.0%, respectively. Iodine affinities before and after defatting of starch, and thermograms of differential scanning calorimetry, indicated that HAG-A and HAG-N starches had a higher proportion of amylose-lipid complex than did NG starch. The amylopectins from HAG-A and HAG-N were similar to NG amylopectin in average chain length (18–19), β -amylolysis limit (β -AL 56–57%), number-

average degrees of polymerization (DP_n 6,000–7,500) and chain length distribution. Very long chains (1–2%) were found in amylopectins from all cultivars. HAG-A amylopectin had a larger amount of phosphorus (214 ppm) than the others. The amyloses from HAG-A and HAG-N resembled NG amylose in DP_n (950–1,080) and β -AL (70–74%). However, HAG-A and HAG-N had a larger number of chains per molecule (NC 2.4–2.7) than NG amylose (1.8) and contained the branched amylose with a higher NC (9.5–10.6) than that of NG amylose (5.8), although molar fractions of the branched amylose (15–20%) were similar.

There are many cultivars of rice, maize, and barley starches differing in amylose content such as waxy, normal, and high-amylose. The effective fractionation of amylose and amylopectin from starches (Takeda et al 1986) and the determination of their molecular structures by recently developed methods revealed that amylopectins from high-amylose cultivars are unique in molecular structure. Indica rice amylopectins contain very long chains in various amounts that are rarely found in waxy and normal Japonica rice amylopectins. The cultivars of Indica rice, which were reported to be high amylose, had an amylose content similar to that of normal Japonica rice. Their apparently higher amylose content is due to a larger amount of very long chains in amylopectin that form a complex with iodine (Takeda et al 1987a). On the other hand, amylomaize starches are actually high amylose (Takeda et al 1989) and their amylopectins are composed of a smaller amount of short chains and a larger amount of long chains than waxy and normal maize amylopectins. Furthermore, amylomaize amylopectins contain very long chains, as observed for Indica rice amylopectin (Takeda et al 1993).

High-amylose barley starches have not been examined from this point of view. The reported amylose contents and structures of amylopectin and amylose were determined from whole starch analyses, that is, without isolation of amylopectin and amylose (Banks et al 1971, 1973; Czuchajowska et al 1998). The often adopted gel-filtration analysis of starch isoamylolyzate is unable to separate the very long chains from amylose. In the present study, amylopectin and amylose were fractionated from two high-amylose barley cultivars and their molecular structures, together with some physicochemical properties of their starches, were characterized and compared with those of normal barley, Indica rice, and amylomaize.

MATERIALS AND METHODS

Materials

Two high-amylose cultivars of barley, high-amylose Glacier A (HAG-A) and high-amylose Glacier N (HAG-N), and a normal cultivar (Normal Glacier, NG), were gifts from American Maize-Prod-

ucts Co. (Hammond, IN). Starch was isolated from barley grains by an alkaline steeping method (0.2% NaOH) and exhaustively defatted by three replicate dissolutions in dimethyl sulfoxide and precipitations with ethanol (Takeda et al 1986). Amylose and amylopectin were fractionated from defatted starch by the method of Lansky et al (1949) with modifications (Takeda et al 1986). The purity of amylose was examined by gel chromatography (Toyopearl HW-75F, Tosoh, Tokyo, Japan) (Takeda et al 1984). β -Limit dextrin (β -LD) from amylose was prepared as described previously (Takeda et al 1987b). Crystalline *Pseudomonas* isoamylase was the product of Hayashibara Biochemical Inc. (Okayama, Japan). β -Amylase was further purified from a commercial product (Sigma Chemical Co., St. Louis, MO) by the method of Marshall and Whelan (1973). All reagents used in this study were of highest grade commercially available.

Physicochemical Analyses

Iodine affinity. Iodine affinity (IA, g/100 g) was determined by the amperometric titration method (Larson et al 1953) with an automatic system (Takeda et al 1987a).

Pasting properties. Pasting properties of starch slurry at a concentration of 9% (w/w) were determined by a Rapid Visco Analyser 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 the rate of 3.0°C/min, maintained at 92.5°C for 15 min, and then cooled to 40°C at the same rate.

Differential scanning calorimetry. Thermal behavior of starch was determined by differential scanning calorimetry (DSC-7, Perkin-Elmer, Norwalk, CT). Starch (15 mg) was weighed in a DSC pan and water was added (30 mg). The pan was sealed and allowed to stand for 12 hr at 4°C. The scanning temperature range was 30–150°C and the heating rate was 5.0°C/min. Water (30 mg) was used for reference.

X-ray diffraction. X-ray diffraction was performed on an X-ray diffractometer (Rotaflex RV-20013, Rigaku Denki Co., Tokyo, Japan) on wet specimens under the conditions described by Hizukuri et al (1988).

Analytical Methods

The blue value, λ_{max} , and β -amylolysis limit (β -AL) were determined as described elsewhere (Suzuki et al 1981, Takeda et al 1983). The number-average degree of polymerization (DP_n) of amylose and amylopectin was determined by a modification of the Park-Johnson method (Hizukuri et al 1981, Takeda et al 1987b). The average chain-length (CL) was determined by the rapid Smith degradation method (Hizukuri and Osaki 1978, Hizukuri et al

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1981) and by hydrolysis with isoamylase (Suzuki et al 1981). The average number of chains per molecule (NC) of amylose was calculated as DP_n/CL . Carbohydrates and reducing sugars were determined by the phenol-sulfuric acid method (Somogyi 1952, Dubois et al 1956) with minor modification (Hizukuri et al 1970). Phosphorus content was determined as inorganic phosphate (Itaya and Ui 1966) after treatment with hot perchloric acid (Allen 1940). Phosphorus linked to C-6 of the glucosyl residue was assayed by using glucose-6-phosphate dehydrogenase after acid hydrolysis (Hizukuri et al 1970). The chain length distribution of amylopectin debranched with isoamylase was determined by gel-permeation HPLC (Hizukuri 1986) and high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (Hanashiro et al 1996). The weight-average degree of polymerization (DP_w) and DP distribution of amyloses and β -LD were determined by gel-permeation HPLC (Hizukuri and Takagi 1984).

Iodine Affinity and Amylose Content

Iodine affinity (IA, g/100 g) and amylose content of barley starches are summarized in Table I. HAG-A and HAG-N starches had higher IA values both before and after defatting and they had a greater increase in IA by defatting than did NG starch. These results indicated that HAG-A and HAG-N starches had more lipid complexing with amylose than did NG starch. The proportion of amylose forming a complex with lipids was 17 and 21% for HAG-A and HAG-N starches, respectively; both were higher than that of NG starch (13%).

The true amylose contents of HAG-A and HAG-N starches, calculated from IA values of defatted starch, amylose, and amylopectin (Table I) were 41.0 and 33.4%, respectively; they were 17.8 and 10.2% higher than that of NG starch (23.2%). On the other hand, the apparent amylose contents, calculated without consideration of amylopectin IA were 40.5% for HAG-A and 37.4% for HAG-N, which is in the range previously reported for some high-amylose cultivars (34.8–45.0%) (MacGregor and Fincher 1993). The difference between the actual and apparent amylose contents (Table I) of the high-amylose barley was small (0–4%) and similar to that reported for normal barley (2.5%) and normal maize (4–5%) (Takeda et al 1988, Takeda and Preiss 1993). A larger difference was observed in amylo maize (8–12%) (Takeda et al 1993). These differences are due to the fact that the high-amylose barley amylopectins had a low iodine affinity, similar to that of normal barley and normal maize amylopectins, and amylo maize amylopectins had a high iodine affinity. These findings suggested that the high-amylose barley amylopectins contained a smaller amount of long chains forming a complex with iodine than did amylo maize amylopectin.

TABLE I
Iodine Affinity (IA) and Amylose Content of Barley Starches^a

Property	HAG-A	HAG-N	NG
Iodine affinity (IA) (g/100 g)			
Defatted starch (A)	8.09	7.48	5.13
Starch (B)	6.73	5.95	4.49
A - B	1.36	1.53	0.64
(A - B)/A	0.17	0.21	0.13
Amylose content (%) ^b	41.0	33.4	23.2
Apparent amylose content (%) ^c	40.5	37.4	25.7

^a High-amylose Glacier A (HAG-A) and N (HAG-N), normal Glacier (NG).
^b $(IA_{\text{defatted starch}} - IA_{\text{amylopectin}}) / (IA_{\text{amylose}} - IA_{\text{amylopectin}}) \times 100$.
^c $(IA_{\text{defatted starch}} / 20) \times 100$.

TABLE II
Thermal Properties^a of Barley Starches

Cultivar ^b	Peak 1				Peak 2			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
HAG-A	56.7	63.0	80.0	10.9	87.2	97.5	104.3	3.7
HAG-N	59.2	64.8	78.4	10.1	88.9	99.8	108.8	3.3
NG	54.0	57.6	75.3	11.5	84.1	97.5	105.6	2.3

^a T_o = onset temperature, T_p = peak temperature, T_c = complete temperature, ΔH = enthalpy change.
^b High-amylose Glacier A (HAG-A) and N (HAG-N), normal Glacier (NG).

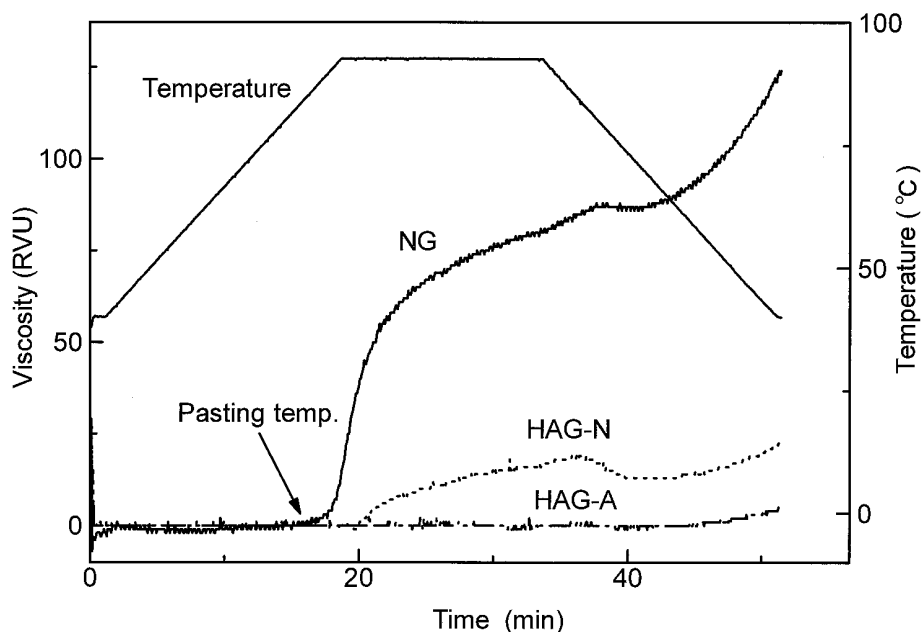


Fig. 1. Barley starch viscosity in Rapid Visco Analyser units (RVU) for high-amylose Glacier A (HAG-A) and N (HAG-N), and normal Glacier (NG). Concentration 9% (w/w).

Pasting Properties

RVA measurements showed that HAG-A starch had no viscosity and HAG-N starch had a much lower viscosity than NG starch (Fig. 1). Similar behavior was reported in barley starches from some high-amylose cultivars (Goering and Dehass 1974, Vasanthan and Bhatti 1996). No or very low viscosity may be due to high amounts of amylose and lipids.

Differential Scanning Calorimetry

DSC thermograms of the barley starches showed two prominent transitions over a similar temperature range (Fig. 2 and Table II).

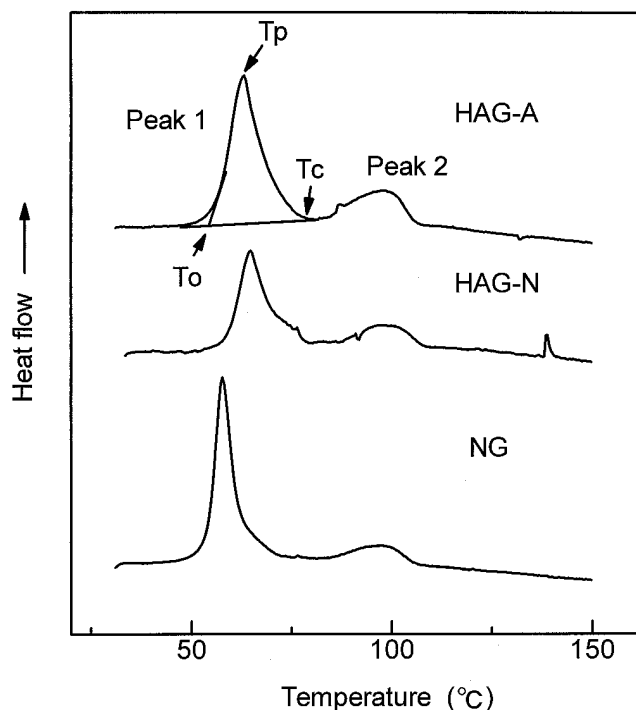


Fig. 2. Differential scanning calorimetry (DSC) thermograms for barley starches. T_o , T_p , and T_c are onset, peak, and complete transition temperatures, respectively, for high-amylose Glacier A (HAG-A) and N (HAG-N), and normal Glacier (NG).

The first (peak 1, 54–80°C) and second (peak 2, 84–109°C) transitions corresponded to melting of starch crystallites which were mainly amylopectin (Morrison et al 1993) and amylose-lipid complex (Kugimiya et al 1980), respectively. In the first transition, HAG-A and HAG-N starches had slightly higher onset (T_o , 56.7–59.7°C), peak (T_p , 63.0–64.8°C), and complete (T_c , 78.4–80.0°C) temperatures, but lower enthalpy (ΔH 10.1–10.9 J/g) than those of NG starch (T_o , 54.0°C, T_p , 57.6°C, T_c , 75.3°C, ΔH 11.9 J/g). In the second transition, HAG-A and HAG-N starches had T_o , T_p , and T_c similar to those of NG starch, but a higher ΔH that appeared to come from a larger amount of amylose-lipid complex. These differences among the cultivars could be caused also by growth conditions (Tester et al 1991), starch granule size (Vasanthan and Bhatti 1996), and amount of starch lipids (Morrison et al 1993).

X-ray Diffraction Pattern

HAG-A and HAG-N starches had the same A-type granules as NG. This finding differed from maize because amylo maize starch showed B-type granules whereas normal maize starch showed A-

TABLE III
Properties of Barley Amylopectins^a

Property ^b	HAG-A	HAG-N	NG
IA (g/100 g)			
Amylopectin	0.66	1.22	0.64
β -Amylolysate	0.24	0.33	0.19
β -Limit dextrin	0.64	0.89	0.44
Blue value	0.07	0.12	0.07
λ_{max} (nm)	556	578	550
CL			
Smith degradation	19	19	18
Isoamylolysis	18	19	18
β -AL (%)	56	57	56
DP_n	7,500	6,200	6,000
Phosphorus			
Organic (ppm) ^c	214	41	45
Linked to C-6, C-6 (ppm)	24	25	7
C-6/Organic (%)	11	61	16

^a High-amylose Glacier A (HAG-A) and N (HAG-N), normal Glacier (NG).

^b Iodine affinity (IA), wavelength maximum (λ_{max}), average chain length (CL), β -amylolysis limit (β -AL), number-average degrees of polymerization (DP_n).

^c Linked to glucosyl residues.

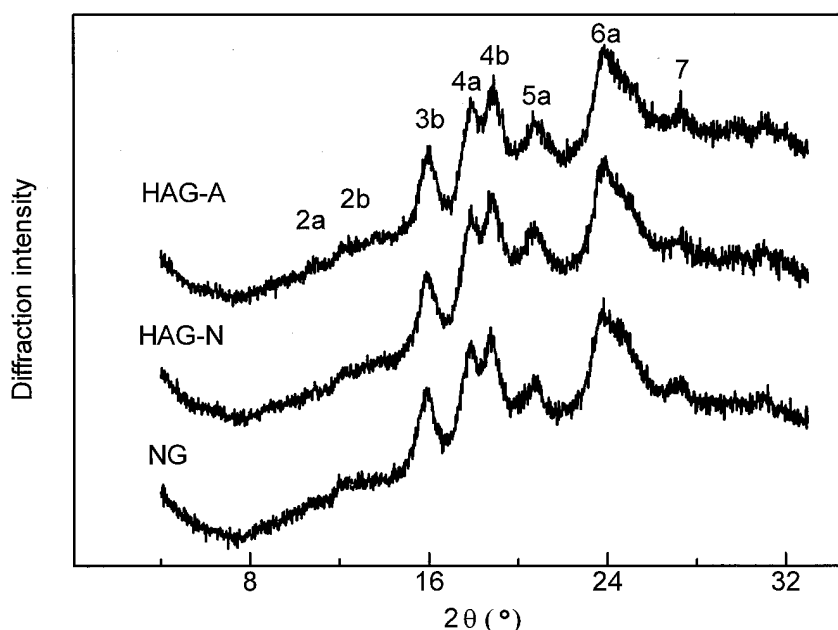


Fig. 3. X-ray diffractograms of barley starches. Numbers indicate interference rings designated by Katz and van Itallie (1930). High-amylose Glacier A (HAG-A) and N (HAG-N), and normal Glacier (NG).

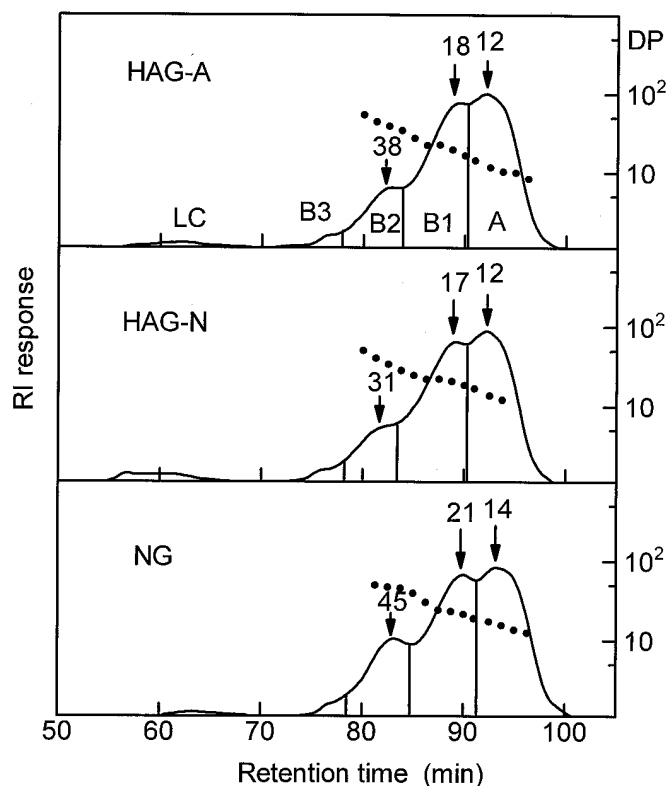


Fig. 4. Gel-permeation HPLC chromatograms of barley amylopectins after isoamylolysis. High-amylose Glacier A (HAG-A) and N (HAG-N), and normal Glacier (NG). Chain fractions LC, B3, B2, B1 and A. Differential refractometry (—); refractive index (RI); degree of polymerization (DP) (●●●).

type granules. The appearance of the same A-type granules in barley starches suggests that HAG-A, HAG-N, and NG amylopectins had similar, but shorter, CL than amylo maize amylopectin starches with long CL showing B-type granules (Hizukuri et al 1983).

Structure of Amylopectin

Table III summarizes the properties of amylopectins from HAG-A, HAG-N, and NG starches. HAG-A amylopectin had a low affinity for iodine (0.66 g/100 g). The value was close to that of NG amylopectin (0.64 g/100 g) but half that of HAG-N amylopectin (1.22 g/100 g). The blue value and λ_{\max} of HAG-A amylopectin were generally the same as those of NG amylopectin but lower than those of HAG-N amylopectin. The IA values of HAG-A and HAG-N amylopectins were less than one third those of amylo maize amylopectins (3.60–4.63 g/100 g) (Takeda et al 1993), suggesting that the high-amylose barley amylopectins contained fewer long chains than amylo maize amylopectins.

HAG-A and HAG-N amylopectins had CL of 18–19, β -AL of 56–57%, and number-average DP (DP_n) of 6,200–7,500, similar to those of NG amylopectin (CL 18, β -AL 56%, DP_n 6,000). HAG-A amylopectin had the largest amount of organic phosphorus (214 ppm), and 11% of the phosphorus was linked to C-6 of the glucosyl residue. The remainders were supposed to be bound to C-3 (Hizukuri et al 1970) because phospholipids were exhaustively removed when the amylopectin was prepared. HAG-N and NG amylopectins had a lower but similar organic phosphorus content (41 and 45 ppm, respectively). The amylopectins significantly differed in the proportion of the phosphorus at C-6 (61 and 16%, respectively). Amylo maize amylopectins had larger amounts of organic phosphorus (75–261 ppm) (Takeda et al 1993) than normal maize amylopectin (15 ppm) (Takeda et al 1988) and half were linked to C-6. It is interesting that both HAG-A and amylo maize amylopectins had similarly high phosphorus contents.

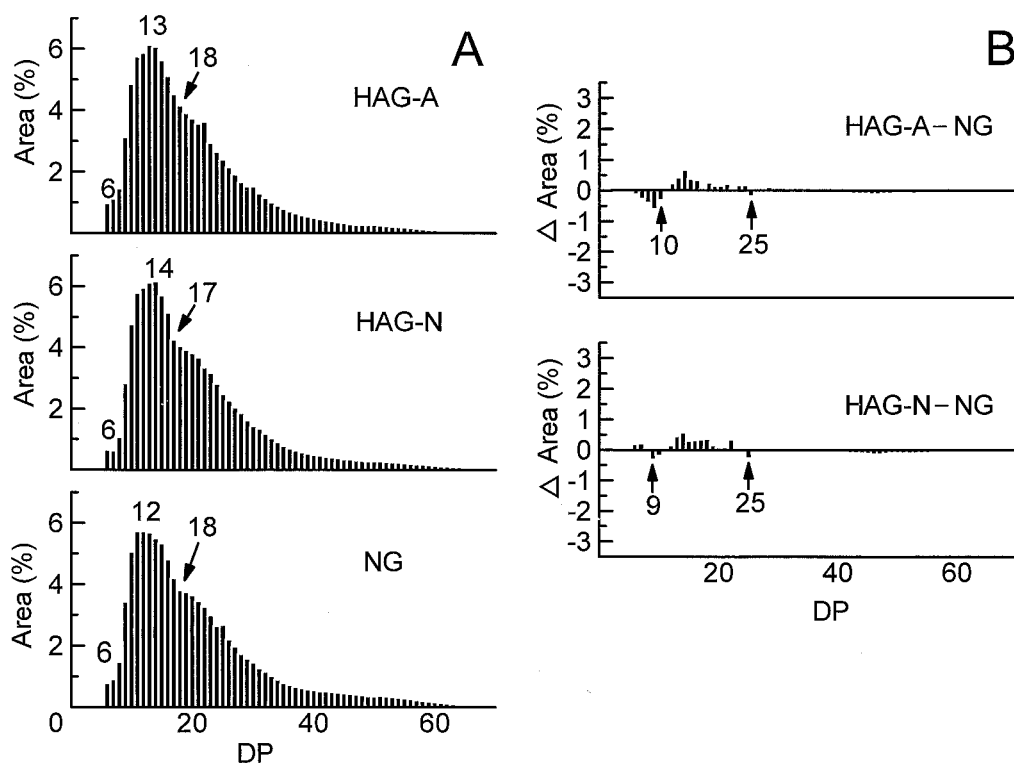


Fig. 5. Chain-length distributions of barley amylopectins (A), and differences between high-amylose and normal cultivars (B). High-amylose Glacier A (HAG-A) and N (HAG-N), and normal Glacier (NG). Differences obtained by subtracting peak areas of HAG-A and HAG-N from that of NG, respectively. Degree of polymerization (DP).

Figure 4 shows the chain-length distribution of barley amylopectins on gel-permeation HPLC after debranching with isoamylase. The chain fractions in order of elution were LC, B3, B2, B1, and A (Hizukuri 1986). Carbohydrate amounts and weight-average chain length (CL_w) of the fractions are summarized in Table IV. HAG-A and HAG-N amylopectins showed chain-length distribution and peak DP similar to those of NG amylopectin. HAG-A, HAG-N, and NG amylopectin had similar carbohydrate amounts and CL_w for each fraction. Each amylopectin had a small amount (1–2% of total) of LC fraction. This fraction seemed to be composed of linear and slightly branched chains that originated from long B-chains with side chains widely spaced, and perhaps long A-chains as observed in Indica rice amylopectins (Takeda et al 1987a) because β -amylolysis decreased IA and the resulting β -LD had a relatively high IA (Table III). The LC proportion and IA value suggested that HAG-N amylopectin had a slightly larger amount of LC than the others.

The high-amylose barley amylopectins significantly differed from amylomaize amylopectins in CL distribution (Takeda et al 1993). The proportion of long chain fractions (LC + B3) of the high-amylose barley amylopectins was 4–5%, which was considerably lower than those of amylomaize amylopectins (25–28%), while the high-amylose barley amylopectins had a higher proportion of short chains (B1 + A, $\approx 70\%$) than those of amylomaize amylopectins ($\approx 40\%$). Therefore, the high-amylose barley amylopectins had considerably lower IA and CL than amylomaize amylopectins (IA 3.60–4.63, CL 29–32) (Takeda et al 1993).

To examine the distribution of short chains in detail, we analyzed the debranched barley amylopectins by HPAEC (Fig. 5A). HAG-A and HAG-N amylopectin peaks DP 13 and 14 had shoulders at DP 17 and 18, respectively, that resembled NG amylopectin. Figure 5B also shows that there was almost no difference in short chain distribution between HAG-A, HAG-N, and NG amylopectins.

These results indicated that there were no significant differences in the fine structure of the barley amylopectins between the high-amylose and normal cultivars. Similar findings were obtained by

Czuchajowska et al (1998), although their experiments were conducted on debranched starch, that is, without isolation of amylopectin, and had not clarified whether the very long side chains existed in amylopectin because the chains were overlapped with amylose fraction on gel chromatography. For maize, however, amylomaize amylopectins significantly differed in the structure from normal maize amylopectins. Thus, it is interesting how amylopectin synthesis differs between high-amylose maize and barley.

Structure of Amylose

Table V summarizes the properties of amyloses from the high-amylose and normal barley cultivars. HAG-A amylose had a slightly lower iodine affinity, blue value, and λ_{max} than the others, while HAG-N and NG amyloses had similar iodine affinity, blue value, and λ_{max} . HAG-A and HAG-N amyloses were similar to NG amylose in DP_n (940–1,080) and showed a DP distribution with a single peak similar to that of NG amylose (Fig. 6). However, the peak DP values of HAG-A and HAG-N amyloses were larger than that of NG amylose. HAG-A and HAG-N amyloses had a larger DP_w (4,080–4,920) than NG amylose (3,440) and a slightly broader distribution judging from DP_w/DP_n values and apparent DP distributions. HAG-A and HAG-N amyloses had CL 350–450 and higher NC (2.4–2.7) than NG amylose (CL 530, NC 1.8), but β -AL (70–74%) and molar fraction of the branched amylose (15–20%) were similar.

The β -LD from the barley amyloses were prepared to elucidate the structure of the branched amylose (Table V). HAG-A and HAG-N β -LD had lower iodine affinities (17.4–17.8) than NG β -LD (19.1), suggesting that the branched amylose of HAG-A and HAG-N had a higher number of chains than that of NG. The DP_n values of the β -LD suggested that the branched amylose of HAG-A was similar in size to that of NG but smaller than that of HAG-N. However, the branched amylose of HAG-A and HAG-N had a wider distribution judging from the DP_w , DP_w/DP_n , and apparent DP distributions of the β -LD. In each amylose, the branched amylose was larger than the linear amylose because the DP_w values of

TABLE IV
Carbohydrate Amounts and Weight-Average Chain Length (CL_w) of Fractions^a of Isoamylase-Debranched Amylopectins

Cultivar ^b	Amount (% of total)					CL_w				
	LC	B3	B2	B1	A	B3	B2	B1	A	$\Sigma(B3 - A)$
HAG-A	1	3	14	38	44	140	45	21	13	24
HAG-N	2	3	15	38	42	140	40	20	12	25
NG	1	3	18	36	42	120	47	23	14	26

^a Chains fractionated into LC, B3, B2, B1 and A, in the order of elution.

^b High-amylose Glacier A (HAG-A) and N (HAG-N), normal Glacier (NG).

TABLE V
Properties of Barley Amyloses^a and Their β -Limit Dextrins (β -LD)

Property ^b	HAG-A		HAG-N		NG	
	Amylose	β -LD	Amylose	β -LD	Amylose	β -LD
Iodine affinity (IA) (g/100 g)	18.8	17.4	20.0	17.8	20.0	19.1
Blue value	1.36	1.57	1.43	1.46	1.44	1.53
λ_{max} (nm)	643	660	646	663	644	664
DP_n	950	1,130	1,080	1,520	940	1,110
DP_w	4,080	4,940	4,920	4,980	3,440	3,400
DP_w/DP_n	4.3	4.4	4.6	3.3	3.7	3.1
Apparent DP distribution ^c	130–15,900	640–16,100	360–19,000	560–16,200	290–12,900	300–11,400
CL	350	120	450	150	530	190
β -AL (%)	70	...	73	...	74	...
NC	2.7	9.5	2.4	10.6	1.8	5.8
Molar fraction (%)						
Branched amylose	20	...	15	...	17	...
Linear amylose	80	...	85	...	83	...

^a High-amylose Glacier A (HAG-A) and N (HAG-N), normal Glacier (NG).

^b Wavelength maximum (λ_{max}), number- and weight average degrees of polymerization (DP_n and DP_w), average chain length (CL), β -amylolysis limit (β -AL).

^c DP values of subfractions (10% w/w) with lowest and highest molecular weight.

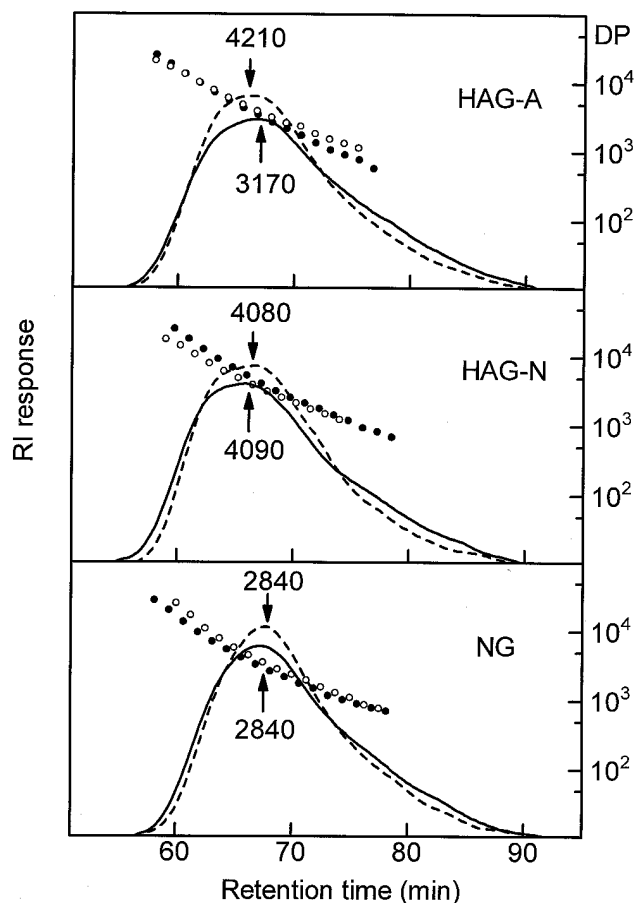


Fig. 6. Gel-permeation HPLC chromatograms of barley amyloses and β -limit dextrins (β -LD). Differential refractometry (—); refractive index (RI); degree of polymerization (DP) (● ● ●) of amylose. Differential refractometry (---) and DP of β -LD (○ ○ ○). Numbers with arrows indicate DP of peaks.

the β -LD were similar to those of amyloses. The branched amyloses of HAG-A and HAG-N might have slightly shorter inner chains than that of NG because their β -LD showed a lower CL. The branched amylose of HAG-A and HAG-N contained 9.5 and 10.6 chains on average, respectively, and was more highly branched than that of NG (5.8 chains). The branched amylose of the high-amylose barley was larger and more branched than that of amylo maize (DP_n 590–610, NC 4.9–6.1) (Takeda et al 1989). Thus, the high-amylose barley amyloses were similar in molecular size to normal barley amylose but contained a branched amylose with more branches. These results differed from those obtained in maize, that is, amylo maize amyloses had smaller molecules than normal maize although the number of chains of branched amylose were similar.

In conclusion, the high-amylose barley had A-type starches like that of the normal barley starch but showed no or very low viscosity. The molecular structure of the amylopectins was similar to that of normal barley amylopectin but the amyloses contained branched amylose with more branches. The high-amylose barley starches actually had a high amount of amylose, like the amylo maize starch, but significantly differed in the fine structure of amylopectin from amylo maize amylopectin. It is of interest to reveal the differentiated biosynthetic mechanism between these amylopectins.

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