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Structure of the amylopectin fraction of amylomaize

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ABSTRACT

The amylopectin fraction (Ap-fraction) from three amylomaize starches (amylose content, 36, 41, and 59%) was fractionated into three amylopectin components (having different molecular size) and a short-chain component by gel-permeation chromatography. The content of the largest amylopectin component was relatively low in a high-amylose maize starch and it had an iodine affinity (i.a., g/100 g) of 1.94–2.50 and average chain length ($\overline{cl}$) of 29–30. Both values increased with decrease of molecular size (i.a. < 7.23, $\overline{cl} < 41$). The proportion of long side chains [weight-average dp ($\overline{dp}_w$) 99–110] increased with decrease of molecular size, whereas that of short side chains ($\overline{dp}_w$ 17–22) decreased. This is the reason why a smaller component had a larger i.a. and $\overline{cl}$. Very long ($\overline{dp}_w$ 400–690) and medium-size ($\overline{dp}_w$ 45–48) side chains were present in similar proportions, regardless of molecular size. The short-chain component was slightly branched (number of chains, 1.9–2.1 on average) and had an i.a. of 8.20–9.57, $\overline{dp}_w$ of 120, and number-average dp of 92–95. A large amount of the short-chain component (27% of the Ap-fraction, by weight) was found in the high-amylose (59%) maize starch.

INTRODUCTION

Several mutants of maize and normal maize contain starches having an apparent amylose content of 0–80%[1–4]. Some normal[5,6], *sugary-2* (B90)[6], and *amylose-extender*[7] maizes have amylose of an approximately similar structure[3], while amylopectins from various varieties[8–13] differ in side-chain distribution and proportions of long and short side chains. Recently, additional very long side chains were found[6] in normal and B90 maize amylopectins fractionated from starches by the method of Lansky et al.[14]. These were absent from waxy maize amylopectins, but were similar to those from some rice amylopectins[15,16]. The very long chains were missed in the experiments using whole starches because they were eluted[5,6] at the same elution position (void volume) as amylose. Amylomaize amylopectin fractions have a high iodine affinity (i.a.), and appear[3,10,17] to contain components having various molecular sizes, including an unusual short-chain component (number-average dp $\sim$ 100). However, details of their structures have not yet been investi-

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gated. We report here the structures of amylomaize amylopectin fractions obtained from completely dispersed starches.

EXPERIMENTAL

Materials.—A laboratory-prepared amylomaize starch (Lab) was the generous gift of Drs. M. Taki and T. Yamada, and commercial amylomaize starches were the products (Hylon and Hylon-7) of Oji National Co., Ltd. (Tokyo). The actual amylose contents of Hylon, Lab, and Hylon-7 starches[7] were 36, 41, and 59%, respectively. The amylopectin fractions (Ap-fractions) were fractionated from the starches, which were defatted by three replications of dissolution and precipitation from Me_2SO (ref 18), by the method of Lansky et al.[14]. After removal of amylose precipitates by centrifugation, supernatant solutions of the Ap-fractions were concentrated to one-tenth volume in a rotary evaporator at 40–45°C, and the Ap-fractions were precipitated by the addition of a 3-fold volume of ethanol, washed with ethanol and ether, and dried under $CaCl_2$. The yields of the Ap-fractions from Hylon, Lab, and Hylon-7 starches (10 g, dry weight) were 5.0, 4.4, and 2.9 g (dry weight), respectively. Beta-amylase was prepared[19] from sweet potatoes and recrystallized from aq ammonium sulfate. Crystalline *Pseudomonas* isoamylase was the product of Hayashibara Biochemical Laboratories Inc. (Okayama). Toyopearl HW-75F and HW-55S were obtained from Tosoh Co., Ltd. (Tokyo). Other chemicals of the highest purity were the products of Wako Pure Chemical Industry Ltd.

Analytical methods.—The iodine affinity (i.a., g/100 g) was determined by the amperometric titration method of Larson et al.[20] with modifications[15]. The blue value was determined as previously described[21]. The number-average dp ($\overline{dp}_n$) was determined by the modified Park–Johnson method[22]. The weight-average dp ($\overline{dp}_w$) was determined[23] by HPLC on three columns of Tosoh TSKgel G6000PW, G4000PW, and G3000PW in series, using a differential refractometer (Tosoh RI-8011) and a low-angle laser-light-scattering photometer (Tosoh LS-8) as detectors. The average chain length ($\overline{cl}$) was determined by the rapid Smith-degradation method[24] and hydrolysis with isoamylase[25]. The number of chains per molecule was calculated as $\overline{dp}_n/\overline{cl}$. The chain-length distribution of the Ap-fractions after isoamylolysis was examined by HPLC on a column of TSKgel G2000SW, using the detectors described above. The beta-amylolysis limit (β-AL) was determined as described previously[22]. Phosphorus was determined[26] as inorganic phosphorus after treatment with hot perchloric acid[27]. Phosphorus at C-6 of the glucosyl residues was determined as described previously[28]. Carbohydrate was determined by the phenol–H_2SO_4 method[29].

RESULTS AND DISCUSSION

Table I summarizes the properties of the amylopectin fractions (Ap-fractions) from Hylon, Lab, and Hylon-7 amylomaize starches, which had amylose contents

TABLE I

Properties of the Ap-fractions from amylomaize starches

Property	Ap-fraction		
	Hylon	Lab	Hylon-7
Iodine affinity (i.a.) (g/100 g)	3.60	4.23	4.63
Blue value	0.427	0.475	0.441
λ_{max} (nm)	573	574	575
Average chain-length ($\overline{cl}$)			
Smith degradation	30	31	32
Isoamylolysis	29	32	32
Beta-amylolysis limit (β-AL) (%)	61	62	61
Phosphorus (ppm)			
Organic	110	75	261
Linked to C-6	54	57	81

of 36, 41, and 59%, respectively, calculated on the basis of the respective amylose and amylopectin i.a.[7,15]. The Ap-fractions had a higher i.a. (3.60–4.63) and blue value (0.427–0.475) than those for normal (i.a., 0.8–1.1; blue value, 0.11, 0.15) and *sugary-2* (B90) (i.a., 3.1; blue value, 0.26) maizes[5,6]. The λ_{max} (573–575 nm) was higher than that (554, 569 nm) for normal maizes, but lower than that (610 nm) for B90 maize. These iodine-binding properties suggest that maize amylopectins differ in structure.

The rapid Smith-degradation and isoamylolysis of each Ap-fraction gave a similar $\overline{cl}$, indicating complete debranching of the Ap-fractions by isoamylase. The $\overline{cl}$ was in the range 29–32, being ca. 1.5-fold those for normal (20, 21.9) and B90 (20) maizes, and Hylon-7 showed the highest $\overline{cl}$ of 32. The β-AL (61–62%) of the Ap-fractions was similar to those of normal (59%) and B90 (60%) maize amylopectins. The organic phosphorus contents were 110, 75, and 261 ppm for Hylon, Lab, and Hylon-7, respectively. The contents were the highest among cereal amylopectins so far examined [normal maize, 15 ppm; rice[15], 8–29 ppm; wheat, 9–20 ppm (unpublished data)], but lower than that (604 ppm) of potato amylopectin[28]. For Hylon, Lab, and Hylon-7, 50, 76, and 31%, respectively, of the phosphorus was linked to C-6 of the glucosyl residues, and the remainder is supposed to be bound to C-3, as in the case of potato amylopectin[28].

Fig. 1 shows the chain-length distributions in gel-permeation HPLC after isoamylolysis. The chains were fractionated into F1–F4, in order of elution (Fig. 1). The Ap-fractions showed a similar $\overline{dp}_w$ for each F2–F3, but a slightly different chain-length distribution. Hylon-7 had a slightly larger proportion of F2 and a rather smaller proportion of F4 compared with the others (Table II). The Ap-fractions differ from normal and B90 maize amylopectins in chain-length distribution and peak dp. The sum of F1 and F2 (25–28%) was larger than that (10–16%) for normal and B90 maizes, while the proportion of F4 (41–44%) was smaller than those (69–70%) of normal and B90[5,6]. The high i.a. of the Ap-fractions is due to

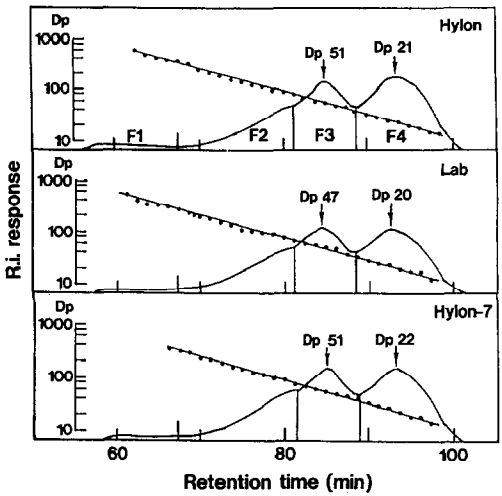

Fig. 1. Gel-permeation HPLC of the Hylon, Lab, and Hylon-7 Ap-fractions debranched with isoamylase: ———, response of the differential refractometer (R.i.); •, Dp.

the relatively large amounts of F1 and F2. The peak dp (20–22) of F4 was larger than those (15–18) for normal and B90 maizes.

Fig. 2 shows gel-permeation chromatograms of the Lab and Hylon-7 Ap-fractions on Toyopearl HW-75F before isoamylolysis. The Ap-fractions were separated into subfractions of large (FL), medium (FM), and small (FS) components (Fig. 2, top), and FS was further fractionated into FSa and FSb by gel-permeation chromatography on Toyopearl HW-55SF (Fig. 2, bottom). The weight proportions of FL, FM, FSa, and FSb were 37, 25, 26, and 12%, respectively, for Lab, and 26, 32, 15, and 27%, respectively, for Hylon-7 (Table III). The Hylon-7 Ap-fraction contained a smaller amount of a large component (FL) than the Lab Ap-fraction, but a larger amount of a small component (FSb). FL had the lowest i.a. (Lab, 2.50; Hylon-7, 1.94); it was lower than those of the parent Ap-fractions and between that of normal and B90 maize amylopectins. The smallest component FSb had the highest i.a. of 8.20–9.57, being about one-half that (~ 20) of amylose. The blue values and λ_{max} showed a tendency similar to the i.a.

TABLE II

Carbohydrate amounts and $\overline{dp}_w$ of F1–F4 of the Ap-fractions debranched with isoamylase

Ap-fraction	Carbohydrate amounts (wt %)				$\overline{Dp}_w$		
	F1	F2	F3	F4	F2	F3	F4
Hylon	4	21	31	44	116	57	22
Lab	3	24	31	42	114	56	22
Hylon-7	2	26	31	41	116	56	23

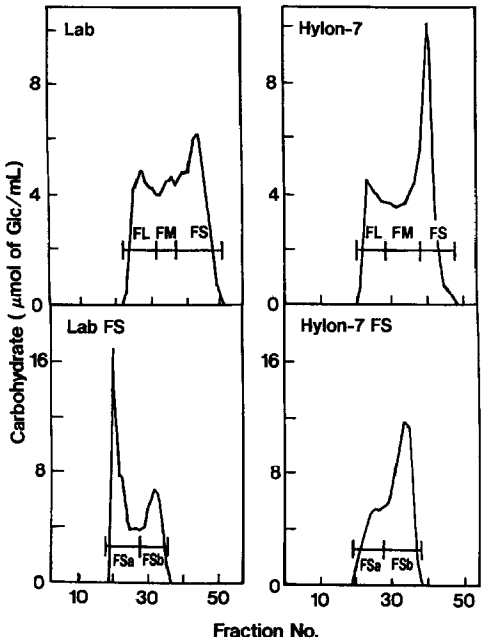

Fig. 2. Gel-permeation chromatograms of the Lab and Hylon-7 Ap-fractions and their subfactions on Toyopearl HW-75F (top) and HW-55SF (bottom).

The $\overline{dp}_w$ and apparent dp distribution of the subfractions (except FL), being excluded by gel-permeation HPLC, were determined (Fig. 3). FM had a $\overline{dp}_w$ of ~ 20000 with a peak dp of 28200 and 22400 for Lab and Hylon-7, respectively

TABLE III

Properties of the subfractions from the Ap-fractions

Property	Lab				Hylon-7			
	FL	FM	FSa	FSb	FL	FM	FSa	FSb
Weight proportion, % of total	37	25	26	12	26	32	15	27
I.a. (g/100 g)	2.50	3.65	4.59	8.20	1.94	4.43	7.23	9.57
Blue value	0.297	0.335	0.367	0.597	0.275	0.370	0.445	0.655
λ_{max} (nm)	570	572	573	578	571	574	574	580
$\overline{Dp}_n$ [a]			2720	95			1330	92
$\overline{cl}$								
Smith degradation	31	32	37	46	29	34	42	49
Isoamylolysis	29	33	34	46	28	33	39	50
β-AL (%)	57	58	57	73	58	57	57	74
External $\overline{cl}$ [b]	19	21	22	36	19	21	25	39
Internal $\overline{cl}$ [c]	10	11	13	9	9	12	15	10
Number of chains ($\overline{dp}_n/\overline{cl}$)			76	2.1			32	1.9

[a] Number-average dp. [b] and [c], Calculated by the equations, $(\overline{cl} \times \beta\text{-AL}/100)+2$ and $[\overline{cl}-(\text{external } \overline{cl})-1]$, respectively.

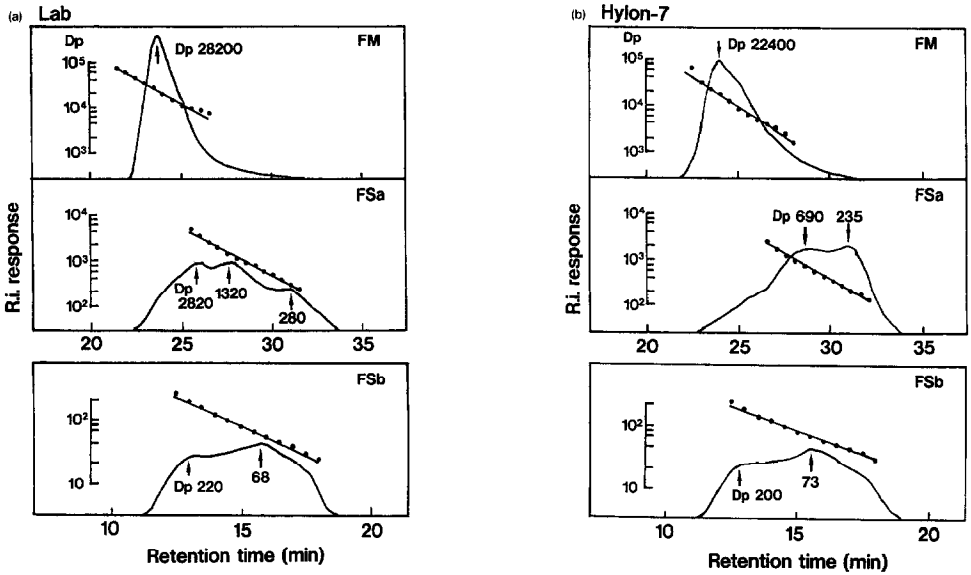

Fig. 3. Gel-permeation chromatograms of the subfractions of the Lab (a) and Hylon-7 Ap-fractions (b) on Tosoh TSKgel G6000PW, G4000PW, and G3000PW in series (top and middle) and on Tosoh TSKgel G2000SW (bottom): ———, response of the differential refractometer (R.i.); •, Dp.

(Table IV). FSa had a $\overline{dp}_w$ of 4250 and 2110 for Lab and Hylon-7, respectively, and Lab and Hylon-7 had three (dp 2820, 1320, and 280) and two (dp 690 and 235) peaks, respectively. The $\overline{dp}_w$ of FSb was 120, and its peak dp values were ~ 210 and ~ 70; its apparent dp distribution was relatively wide (dp_w 40– ~ 350). The $\overline{dp}_n$ of FSa was 2720 and 1300 for Lab and Hylon-7, respectively, being in the range for amyloses[16,22,30] (800–4920), and FSb had a $\overline{dp}_n$ of 92–95 (Table III). The $\overline{dp}_n$ of the large components was not determined because adequate amounts of the specimens were not available. The ratio $\overline{dp}_w/\overline{dp}_n$ of FSb was ~ 1.3, indicating a narrow molecular-weight distribution. These results indicate that the Ap-fractions consist of several components with various sizes. A similar short-chain component of amylomaize starches was suggested previously[3,10].

TABLE IV

$\overline{Dp}_w$ [a] and apparent dp distributions of FM, FSa, and FSb

Property	Lab			Hylon-7		
	FM	FSa	FSb	FM	FSa	FSb
$\overline{Dp}_w$ [a]	21200	4250	120	19200	2110	120
$\overline{Dp}_n$		2720	95		1330	92
$\overline{Dp}_w / \overline{dp}_n$		1.56	1.26		1.59	1.33
Apparent dp distribution	10600–47400	500–23300	40–370	7230–42600	230–13400	40–330

[a] Weight-average dp.

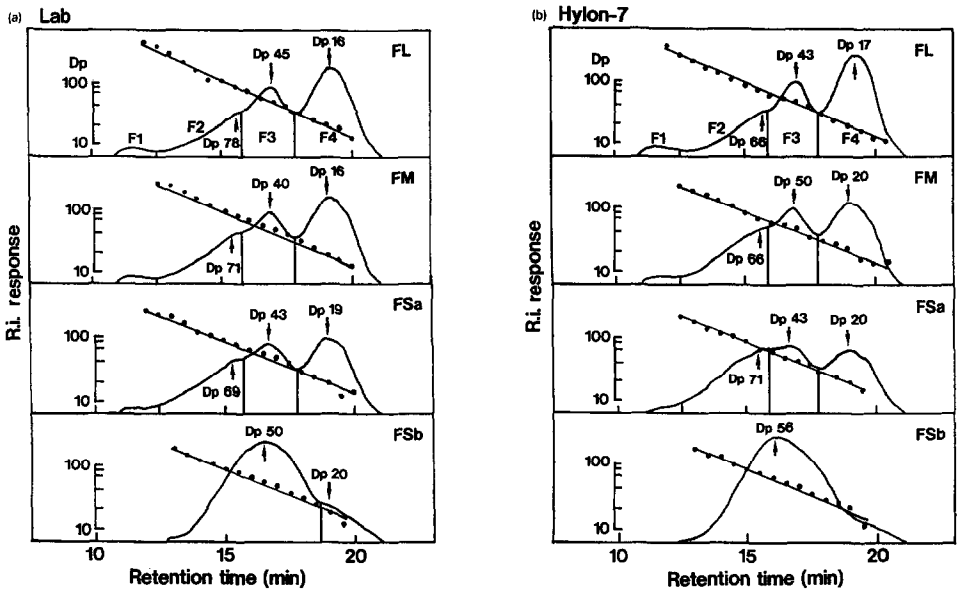

Fig. 4. Gel-permeation HPLC of the subfractions of the Lab (a) and Hylon-7 Ap-fractions (b) debranched with isoamylase; ———, response of the differential refractometer (R.i.); •, Dp.

FL appears to have a similar size to other amylopectins, judging from gel-permeation chromatograms, but its $\overline{cl}$ (29–30) was much larger than those of normal (20, 21.9) and B90 (20) maize amylopectins. The smaller component showed a larger $\overline{cl}$, and FSb had the largest $\overline{cl}$ (46–50). The β-AL of the components, except FSb, was 57–58%, slightly lower than the parent Ap-fractions (61–61%), due to the fact that the parent Ap-fractions contained FSb of a high β-AL (73–74%). Both the external and internal $\overline{cl}$ of the components, except for FSb, increased with decrease of molecular size. These $\overline{cl}$ values were larger than those of normal and B90 maize amylopectins (external and internal $\overline{cl}$, 14–15 and 5–6, respectively). FSa had, on average, 32–76 chains per molecule. FSb had a very small number of chains per molecule (~ 2), implying that FSb was a mixture of linear chains, as suggested previously[3,10], and slightly branched chains. FSb did not form a complex with 1-butanol, due to the small molecular size, but formed a complex with iodine and gave a high i.a. Similar short-chain components have not been found in starches from other maizes and plants.

Fig. 4 shows the chain-length distribution of the components in gel-permeation HPLC after debranching by isoamylase. FL, FM, and FSa showed an elution profile with two peaks (dp 16–20 and 40–50) and a shoulder (dp 66–78), similar to normal and B90 maize amylopectins (dp 15–18, 40–47) although the shoulder is not clear. In this respect, FL, FM, and FSa are typical amylopectin components. The chains were separated into four fractions, F1–F4 in order of elution. F1–F4 showed a $\overline{dp}_w$ of 400–690, 99–110, 45–48, and 17–22, respectively (Table V). The

TABLE V

Carbohydrate amounts and $\overline{dp}_w$ of F1–F4 of FL, FM, and FSa debranched with isoamylase

	$\overline{Dp}_w$				Carbohydrate amount (%)								
					By weight					By mole			
	F1	F2	F3	F4	F1	F2	F3	F4	F4/F3	F1	F2	F3	F4
Lab													
FL	520	110	48	21	3	18	31	48	1.55	~0.2	5	18	77
FM	690	108	47	18	3	22	31	44	1.42	~0.1	6	20	74
FSa	410	101	46	17	2	27	30	40	1.33	~0.2	8	20	72
Hylon-7													
FL	490	99	47	20	3	19	28	50	1.79	~0.2	6	18	76
FM	510	103	46	22	3	27	29	41	1.41	~0.2	9	23	68
FSa	400	103	45	22	2	35	29	34	1.17	~0.2	13	26	61

$\overline{dp}_w$ values of F3 and F4 are comparable with those for the corresponding fractions of normal ($\overline{dp}_w$ 46–47 and 17–18, $\overline{dp}_n$ 44 and 16), waxy[31] ($\overline{dp}_w$ 51 and 18), and B90 ($\overline{dp}_n$ 40 and 15) maize amylopectins. The carbohydrate proportions, on a weight basis, of F1–F4 were 2–3, 18–35, 28–31, and 34–50%, respectively. The sum of the F1 and F2 proportions (21–37%) is higher than those (10–12%) of normal maize amylopectins[5], and this is the reason why the amylomaize amylopectin components had a high i.a. The B90 amylopectin has a lower proportion (16%) but a relatively higher i.a. (3.1), due to a higher proportion (~10%) of very long chains (F1) having a greater affinity for iodine. These components showed a higher proportion of F3 than normal (19–20%) and B90 (15%) maize amylopectins, but a lower proportion of F4 (normal 69–70, B90 69%). The ratios F4/F3 were 1.17–1.79. The values are similar to those published for amylomaize[13,31] (0.8, 1.1), but lower than those for normal (2.9, 3.5) and mutant[6,13,31] (waxy 2.9, 3.9; *sugary-2* 2.7, 4.6; *dull* 4.0) maizes. A smaller amylopectin component showed a higher proportion of F2, but a lower proportion of F4, similar to their proportions on a molar basis. This is the reason for its higher i.a. FSb showed a unique elution profile with a peak of dp 50–56, and differed markedly from amylopectin.

Thus, the Ap-fractions, obtained from the completely dispersed, defatted starches of the amylomaizes, were composed of amylopectin (FL-FSa) and a short-chain component (FSb). The amylopectin was poorly branched compared with normal and *sugary-2* (B90) maize and other amylopectins, and contained a large proportion of long chains (F2) and a small proportion of short chains (F4). This is probably due to a deficiency of branching enzyme IIb[32] in amylomaize which preferentially transfers short chains[33]. The amylopectin contained components with various sizes and a slightly different chain-length distribution. The proportion of the largest component was lower in the amylomaize starches than in other starches, whereas medium and small components were predominant. A smaller component had a larger amount of long chains and a smaller amount of short chains, resulting in a higher i.a. The short-chain component (FSb) was a

mixture of linear (short-chain amylose) and slightly branched molecules, and was most evident in the high-amylose maize starch (Hylon-7). Such a short-chain component has not been detected in other starches. This and previous[5,6,31] results indicate that the amylopectins from waxy, normal, *sugary-2*, and *amylose-extender* maizes differ significantly in details of their fine structure.

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