Structure of Amylomaize Amylose

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ABSTRACT

The molecular structures of three varieties of amylomaize amyloses, and their β-limit dextrins were analyzed by chemical end-group determination, high-performance liquid-gel chromatography with a small-angle laser-light-scattering photometer and differential refractometer and enzymic analyses. The results suggested that the structures of amyloses from amylomaize starches with apparent amylose contents of 48, 54, and 68% closely resembled each other. The amyloses were small molecules of 690–740 number-average degrees of polymerization (dp_n) and 1,810–1,990 weight-average dp_w, with chain lengths of 215–255, and their apparent dp distributions were 200–9,000. They comprised large branched molecules, with 5–6 chains on average, and small unbranched molecules in an almost equimolar ratio.

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Amyloses of different origins are unique as to molecular size, molar fractions of branched and unbranched molecules, and chain length and number of chains of the branched molecule (Y. Takeda et al 1987b), but no differences in the molecular structures of amyloses among different varieties of rice (Y. Takeda et al 1986a) and sweet potato (Y. Takeda et al 1986b) have been found. These findings may imply that the inherent structure of an amylose depends on the plant species. However, amylomaize amylose has been suggested to be a smaller molecule than normal maize amylose (Greenwood and Mackenzie 1966, Adkins and Greenwood 1969, Banks et al 1974). Y. Takeda et al (1988) recently characterized the structure of normal maize amylose. In this study we analyzed the fine structures of amyloses from amylomaize starches with apparent amylose contents of 48, 54, and 68% to clarify whether the molecular structure depends on the variety.

MATERIALS AND METHODS

Materials

A laboratory-made (Lab) amylomaize starch, which was prepared from kernels steeped in an alkaline solution, was obtained from M. Taki and T. Yamada. Commercial starches (Hylon and Hylon-7) were the products of Oji National Co., Ltd. The iodine affinities of the exhaustively defatted (Y. Takeda et al 1987a) Lab, Hylon, and Hylon-7 starches were 10.5, 9.31, and 13.4 g/100 g, respectively. Their apparent amylose contents, which were calculated by the iodine affinities of the starches and amyloses wihtout consideration of those of their amylopectins, were 54, 48, and 68%, respectively. Amyloses were fractionated from the starches, which were defatted by three replications of dissolution and precipitation from dimethyl sulfoxide, and were purified by the method of Y. Takeda et al (1984). The yields of the amyloses from 10 g (dry weight) of the Lab, Hylon, and Hylon-7 starches were 3.5, 2.8, and 4.3 g (dry weight), respectively. The yields (58–65%) for the apparent amylose contents were low, but those for the actual amylose contents (39, 36, and 59%, respectively) with consideration of the iodine affinities (4.23, 3.60, and 4.63 g/100 g, respectively) of their amylopectins were reasonably high (90, 78, and 73%, respectively). β-Limit dextrins were prepared from the amyloses by the method of Y. Takeda et al (1987b). β-Amylase was prepared (Takeda and Hizukuri 1969) from sweet potatoes and recrystallized from aqueous ammonium sulfate to improve its stability during storage. Crystalline *Pseudomonas* isoamylase and *Klebsiella* pullulanase were the products of Hayashibara Biochemical Laboratories Inc. Toyopearl HW-75F was obtained

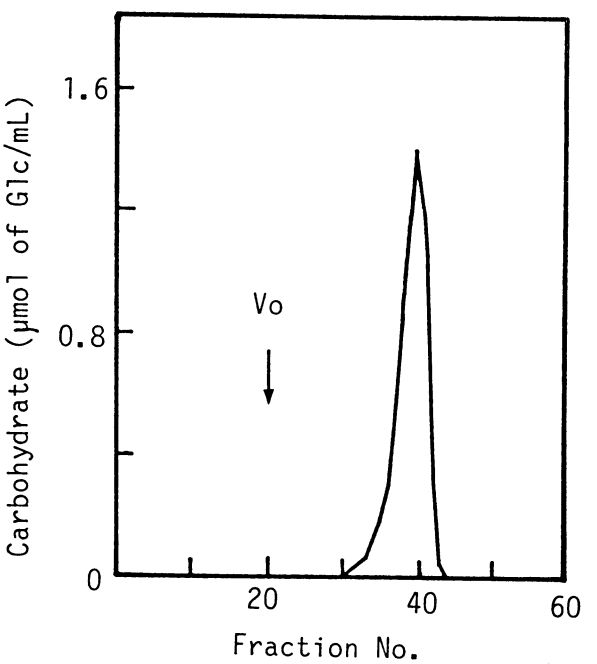

Fig. 1. Gel filtration curve for Hylon amylose on Toyopearl HW-75F. The conditions were as reported (Takeda et al 1984). The void volume of the column (**Vo**) was determined from the elution volume for potato amylopectin.

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from Tosoh Co., Ltd. (Tokyo). Other chemicals were of the highest purity available commercially.

Methods

The purity of the amyloses was examined by gel-permeation chromatography on Toyopearl HW-75F (Y. Takeda et al 1984). The iodine affinity was determined at 25° C by the amperometric titration method of Larson et al (1953) with modifications (Y. Takeda et al 1987a). The blue value was determined by the method of C. Takeda et al (1983). The limiting viscosity number [η] was determined in 1M KOH at 22.5° C with an Ostwald viscometer. The number-average degrees of polymerization (dp_n) of the amyloses and β-limit dextrins were determined by the modified Park-Johnson method (Hizukuri et al 1981). The weight-average degrees of polymerization (dp_w) and dp distribution were determined by high-performance gel chromatography (HPLC) on three columns, TSK-gel G6000PW, G4000PW, and G3000PW (Tosoh Co., Ltd.), in series using a differential refractometer (Tosoh, RI-8011) and a low-angle laser-light scattering photometer (Tosoh, LS-8) as detectors (Hizukuri and Takagi 1984). The sedimentation study was carried out with a Hitachi ultracentrifuge (model 282) at 57,000 rpm at 23° C. Specimens of 2.5, 5.0, 7.5, and 10 mg/ml in 90% dimethyl sulfoxide were used for determination of the limiting sedimentation velocity.

The average chain length (CL) was determined by the rapid Smith-degradation method (Hizukuri et al 1981) with minor modifications (Y. Takeda et al 1984). The number of chains per molecule was calculated as dp_n/CL. The β-amylolysis limit was determined by the method of Suzuki et al (1981). Phosphorus was determined (Itaya and Ui 1966) as inorganic phosphorus after treatment with hot perchloric acid (Allen 1940). Carbohydrate was determined by the anthrone-sulfuric acid method (Dische 1962). The treatment of amylose with isoamylase was carried out as reported by Hizukuri et al (1981).

RESULTS AND DISCUSSION

The amylomaize amyloses were fractionated and purified by the gentle boiling method recently developed, avoiding conventional autoclaving for cereal starch, which markedly degrades amylose. The method appears to be most suitable and causes the least degradation during the preparation of cereal amyloses, because negligible change of the molecular weight distribution of amylose was observed during the boiling (Y. Takeda et al 1986a). Figure 1 shows the gel-filtration profile of the amylomaize amylose obtained from a commercial starch with a 48% amylose content (Hylon). The single elution peak and absence of carbohydrate at the void volume indicated that the amylose was free of amylopectin. The high purity of the amyloses obtained from a laboratory-made starch with a 54% amylose content (Lab) and a commercial starch with a 68% amylose content (Hylon-7) was confirmed on the basis of the same criteria. The preparation of pure amylomaize amyloses required complete removal of lipid from the starches, for which dissolution and precipitation of the starches from dimethyl sulfoxide was repeated three times.

Table I summarizes the properties of the Lab, Hylon, and Hylon-7 amyloses, and it can be seen that these amyloses are structurally similar. The amyloses showed normal iodine-staining properties, similar to other amyloses (Y. Takeda et al 1987b, 1988), but they may have slightly lower iodine affinities than those of the amylomaize amyloses reported previously (Adkins and Greenwood 1969). The amylomaize amyloses contained detectable amounts of phosphorus, as low as those of root, bulb, and tuber amyloses (2–9 ppm) (Suzuki et al 1981, 1986; C. Takeda et al 1983, 1987; Y. Takeda et al 1984). The limiting viscosity numbers [η] of the amylomaize amyloses were 136–147 ml/g, which are close to those (122–155 ml/g in 0.15M KOH) of amyloses from amylomaize starches with amylose contents of 57–75% (Adkins and Greenwood 1969). The number (dp_n) and weight (dp_w) average degrees of polymerization of the Lab, Hylon, and Hylon-7 amyloses were in the ranges of 690–740 and 1,810–1,990, respectively. These values are lower than those ([η], 166–183 ml/g; dp_n, 930–990; dp_w, 2,270–2,550) of normal maize amylose (Y. Takeda et al 1988). Thus, the amylomaize amyloses are smaller molecules than normal maize amylose, as reported previously (Adkins and Greenwood 1969), and are the smallest among 11 kinds of amyloses from cereal, nut, root, bulb, and tuber starches examined so far (Hizukuri et al 1981, 1988; Hizukuri and Takagi 1984; Suzuki et al 1986; C. Takeda et al 1983, 1987; Y. Takeda et al 1984, 1986a,b, 1987b, 1988).

The amylomaize amyloses showed similar dp distribution curves with maxima at 1,540-1,700 dp and shoulders at 700–830 dp (Fig. 2). The higher slope in the ascending versus descending portion of curves suggests that the larger molecules are more spherical compared with the smaller ones, probably due to their higher degree of branching. The whole elution profiles resembled that of normal maize amylose (Y. Takeda et al 1988). Figure 3 shows the sedimentation profile of the Hylon-7 amylose, of which the limiting sedimentation coefficient was 1.43×10^{-13} sec in 90% dimethyl sulfoxide at 23° C. The asymmetrical profile with more abundant small molecules than large molecules is consistent with the HPLC gel chromatogram. Other amyloses appear to show a similar pattern to that of Hylon-7 judging from their HPLC profiles. The dp_w/dp_n values of the amylomaize amyloses were close to those of normal maize amylose (2.44–2.66, Y. Takeda et al 1988) and rice amylose (2.64–3.39, Y. Takeda et al 1986a), but higher than those of potato (1.29) and sweet potato (1.31) amyloses (Hizukuri and Takagi 1984). The apparent dp distributions of the amylomaize amyloses ranged from 200 to 9,000. These results indicate that the amylomaize amyloses have relatively broad molecular weight distributions, as do normal maize amylose (Y. Takeda et al 1988) and rice amylose (Y. Takeda et al 1986a).

The amylomaize amyloses had lower CL values than normal maize amylose (CL, 295–335) (Y. Takeda et al 1987b, 1988) and those of other sources (270–670) (Hizukuri et al 1981, 1988; Suzuki et al 1986; C. Takeda et al 1983, 1987; Y. Takeda et al 1984, 1986a,b, 1987b, 1988). The dp_n/CL ratios indicate that the amylomaize amyloses are molecules with approximately three chains on average, similar to normal maize amylose (2.8–3.4 chains, Y. Takeda et al 1987b, 1988) and others (3.5–4.9) except potato (7.3) and sweet potato (10–13) amyloses (Hizukuri et al 1981; Suzuki et al 1986; C. Takeda et al 1983, 1987; Y. Takeda et al 1984, 1986a,b, 1987b, 1988). The β-amylolysis limits of the amylomaize amyloses were 75–78%, which is within the reported range (Greenwood and Mackenzie 1966, Adkins and Greenwood 1969) but lower than that (81–84%) of normal maize amylose (Y. Takeda et al 1988). Simultaneous hydrolysis of the amyloses with β-amylase and pullulanase led to complete degradation, suggesting

TABLE I
Properties of Amylomaize Amyloses

Property	Laboratory-made	Commercial	
		Hylon	Hylon-7
Iodine affinity, g/100 g	19.4	19.4	19.6
Blue value	1.35	1.39	1.32
λ_{max}, nm	650	650	645
[η], ml/g	136	147	139
Phosphorus, ppm	4	3	5
dp_n[a]	710	740	690
dp_w[b]	1,870	1,810	1,990
dp_w/dp_n	2.63	2.45	2.88
Apparent dp distribution[c]	230–8,070	210–7,880	270–8,940
Chain length (CL)	245	255	215
Number of chains (dp_n/CL)	2.9	2.9	3.2
β-Amylolysis limit, %	78	76	75
Treated with isoamylase			
dp_n	290	310	250
Number of chains	1.18	1.22	1.16
β-Amylolysis limit, %	92	88	92

[a] Number-average degrees of polymerization.
[b] Weight-average degrees of polymerization.
[c] The dp values of the subfractions (10% amylose by weight) having the lowest and highest molecular weights.

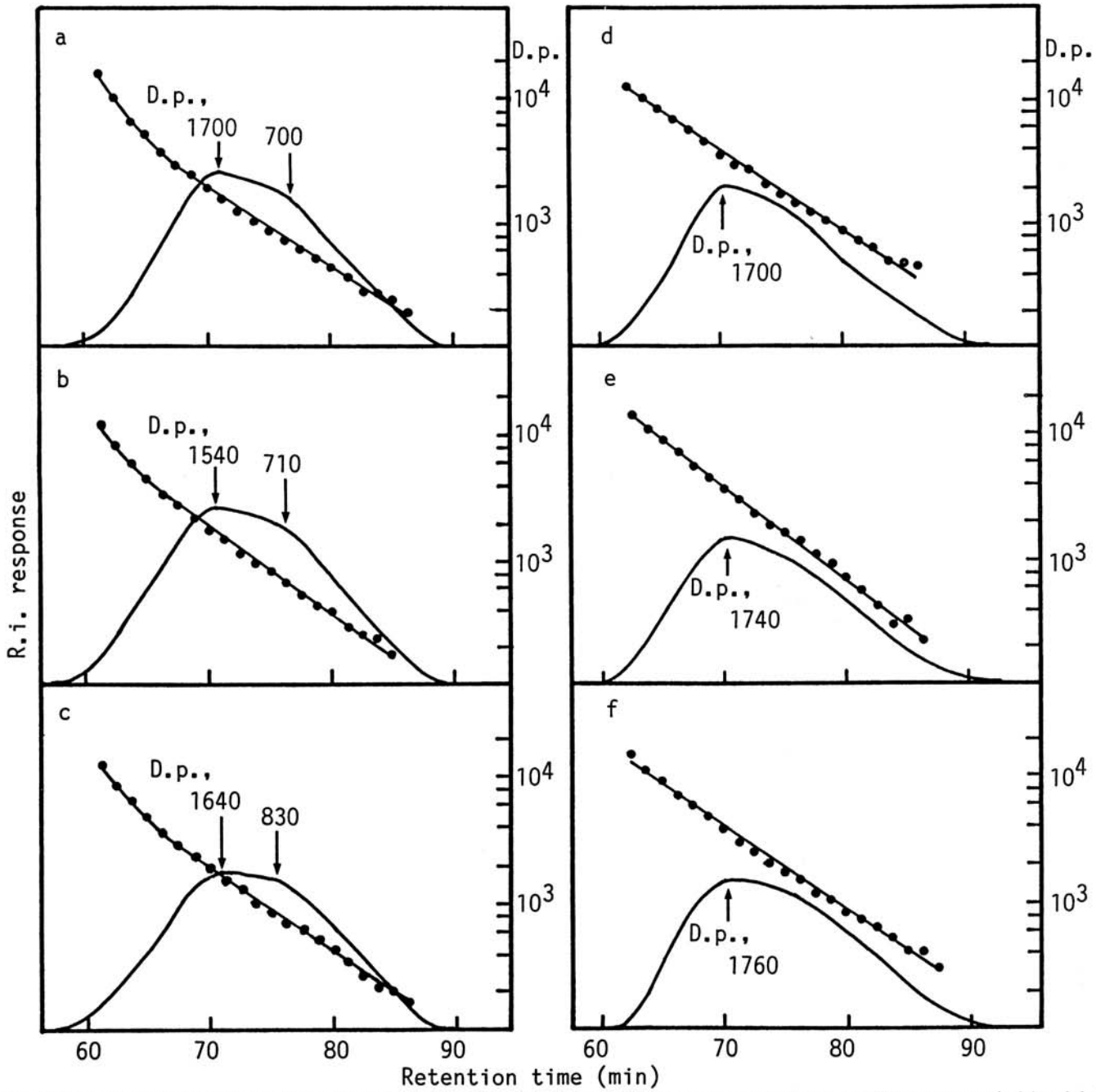

Fig. 2. High-performance liquid chromatography elution curves for amylomaize amyloses (**a**, **b**, and **c**: Lab, Hylon, and Hylon-7, respectively) and their β-limit dextrins (**d**, **e**, and **f**: Lab, Hylon, and Hylon-7, respectively). The conditions were as reported (Hizukuri and Takagi 1984). Response of the differential refractometer (——); degrees of polymerization (•).

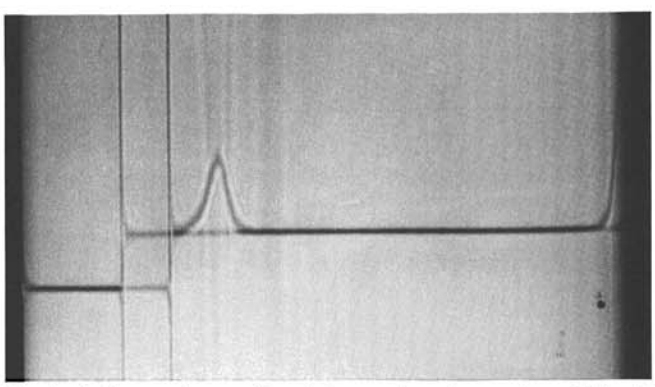

Fig. 3. Sedimentation profile of the amylomaize amylose (Hylon-7). Photograph was taken 1.5 hr after the maximum speed (57,000 rpm) was reached. The direction of sedimentation was from left to right. The concentration of the amylose was 7.5 mg/ml.

TABLE II
Properties of β-Limit Dextrins from Amylomaize Amyloses

Property	Laboratory-made	Commercial	
		Hylon	Hylon-7
Iodine affinity, g/100 g	17.7	18.3	17.0
Blue value	1.31	1.34	1.30
λ_{max}, nm	648	650	642
dp_n[a]	610	670	590
dp_w[b]	1,400	1,530	1,620
dp_w/dp_n	2.30	2.28	2.75
$dp_n/dp_{n\ (amylose)}$	0.86	0.91	0.86
$dp_w/dp_{w\ (amylose)}$	0.75	0.85	0.81
Apparent dp distribution	220–4,040	140–4,590	220–4,830
Chain length	111	135	98
Number of chains	5.5	4.9	6.1

[a] Number-average degrees of polymerization.
[b] Weight-average degrees of polymerization.

TABLE III
Molar Fractions of Branched and Unbranched Molecules in Amylomaize Amyloses

	Laboratory-made	Commercial	
		Hylon	Hylon-7
Branched molecule[a]	0.42	0.47	0.43
Unbranched molecule	0.58	0.53	0.57

[a] $(NC_{amylose} - 1)/(NC_{\beta\text{-limit dextrin}} - 1)$, where NC = average number of chains.

that the chains are linked through α-(1→6) linkages as reported for other amyloses (Hizukuri et al 1981, Y. Takeda et al 1987b). Isoamylolysis decreased the dp_n of the amyloses and increased their β-amylolysis limits (Table I). However, the incomplete β-amylolysis and numbers of chains of the isoamylolysates indicate the presence of some branches that are unhydrolyzable by isoamylase in the amyloses as observed for other amyloses (Hizukuri et al 1981; Suzuki et al 1986; C. Takeda et al 1983, 1987; Y. Takeda et al 1984, 1986a,b, 1988).

Table II summarizes the properties of β-limit dextrins derived from branched molecules in the amylomaize amyloses. The Lab, Hylon, and Hylon-7 dextrins were similar and showed slightly lower iodine affinities, blue value, and λ_{max} than those of the parent amyloses, as observed in the cases of other amyloses (Y. Takeda et al 1987b, 1988). The amylomaize dextrins showed similar dp distributions and dp values of 1,700–1,760 at the maximum elution peaks (Fig. 2d–f). The dp_n and dp_w of the dextrins were in the ranges of 590–670 and 1,400–1,620, respectively. These values are lower than those (dp_n, 790 and 850; dp_w, 2,700 and 3,000) of normal maize dextrin (Y. Takeda et al 1988). The amylomaize dextrins had lower dp_w/dp_n values than normal maize dextrin (3.4 and 3.5) (Y. Takeda et al 1988). The apparent dp distributions of the amylomaize dextrins were in the range of 150–5,000. These results suggest that the amylomaize amyloses comprise smaller branched molecules, with a narrower molecular weight distribution, than normal maize amylose.

The amylomaize β-limit dextrins had lower CL values than normal maize dextrin (CL 140–150; Y. Takeda et al 1987b, 1988) and five or six chains on average, suggesting that the amylomaize amyloses comprise branched molecules with shorter inner chains and similar numbers of chains, compared with normal maize amylose (Y. Takeda et al 1987b, 1988). The dp_n and dp_w of the dextrins were a little lower than those of the parent amyloses. The molar fractions of branched and unbranched molecules, which were calculated from the numbers of chains of an amylose and its β-limit dextrin, were in the range of 42–47 and 58–53%, respectively (Table III).

In conclusion, the molecular structures of the amylomaize amyloses were similar and no significant varietal difference was found, regardless of the apparent amylose contents (48, 54, and 68%), as previously noticed on rice amyloses (Y. Takeda et al 1986a). They had smaller molecular sizes and shorter chain lengths than those of normal maize amylose. They comprised nearly equal numbers of large branched and small unbranced molecules, and the branched molecules had five or six chains on average.

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