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Note

Examination of the structure of amylopectin molecules by fluorescent labeling

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Abstract

Amylopectin molecules from rice, maize, sweet potato and potato were examined by fluorescent labeling followed by gel-permeation HPLC. The number-average degree of polymerization (dp_n) was determined to be in range of 9600–15,900. The molar-based distribution revealed the presence of three molecular species, large (dp_n 13,400–26,500), medium (4400–8400) and small (700–2100). Their molar proportions differed by plant origin. The large species was a major component (43–63% by mole). A relatively large amount of the medium (16–28% by mole) and small (19–38%) species was found although their weight proportion was small (8–15, 1–4%, respectively). The three species from waxy rice amylopectin had a similar chain-length distribution and also a similar size-distribution of C chains. These results suggested that the three species were basically similar in cluster structure but different in number of clusters per molecule.

Keywords: Starch; Granule; Amylopectin; Fluorescent labeling; Gel-permeation HPLC

Starch contains small, linear and slightly branched molecules of amylose and large, highly branched molecules of amylopectin, and their molecular structures are distinct by plant origin.[1,2] Recently, fluorescent labeling of the reducing terminal of amylose and amylopectin unit-chains followed by gel-permeation HPLC (F-GPC) enabled determination directly of their molar-based distributions and number-average degree of polymerization (dp_n),[3,4] and revealed more detailed molecular structure of amylose and amylopectin than by conventional weight-based determinations.

The molar-based distribution showed that amylose was composed of several molecular species of different size, and their molar proportion differed by botanical source.[3] Each amylopectin cluster, which is the smallest unit of amylopectin,[5,6] was composed of a different number of chains by plant origin. The C chain of amylopectin, which is the only chain having the reducing terminal residue per molecule,[7] had a similar size to other unit chains of amylopectin.[4] The dp_n of amylose and number-average chain length (cl_n) of amylopectin determined by F-GPC were in agreement with those by colorimetric method.[8–10] We here examined the structure of amylopectin molecules by F-GPC and determined the dp_n of amylopectin, which was difficult to determine by conventional method[8,11] with good accuracy.

Figure 1 shows the molar-based distribution of amylopectin from maize, rice, sweet potato and potato. All the amylopectins showed three molecular species differing in size, large, medium and small, although the weight-based distribution showed a single peak and was similar among the different plant sources. The large species was predominant, and the medium and small species varied in amount with plant source. The peak-top dp of the large, medium and small species was 13,000–26,000, 3400–8000 and 430–1500, respectively. Starch isolation and preparation of amylopectin was carried out under a mild condition to minimize a possible degradation of amylopectin. Also, under the labeling condition, no degradation had been observed for amylose[3] or debranched amylopectin.[4] Therefore the medium and small species of amylopectin were not artifacts generated during the labeling procedure.

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The dp_n of amylopectin, calculated from peak areas of both responses of fluorescent and refractive index detectors (see Section 1), was in the range of 9600–15,900 (Table 1). Waxy maize had the smallest dp_n whereas normal maize the largest. The dp_n value was reproducible within 5% error, and a similar dp_n was obtained when various concentrations (10–60 mg/mL as a sample solution) of waxy rice amylopectin were employed. The recovery of carbohydrate was higher than 95% after F-GPC. Thus, dp_n of amylopectin can be determined by F-GPC, which is more suitable than a conventional, modified Park–Johnson method.[8,11]

The dp_n of the large, medium and small species, fractionated as indicated in Fig. 1, was 13,400–26,500, 4400–8400 and 700–2100, respectively (Table 1). The value of the small species appeared to be poor in accuracy due to weak response of a refractive index detector. Each species differed in size with botanical source. The large and medium species of normal maize were largest, and the medium species of waxy rice and the small species of japonica rice were smallest. The dp_n of each medium species was about one-third that of respective large species. The proportion of the large species was 43–63% by mole but 83–90% by weight. The medium and small species showed a molar proportion of 16–28 and 19–38%, respectively. These species, however, showed a small amount by weight, 8–15 and 1–4%, respectively, and especially the small species was almost negligible.

Labeled waxy rice amylopectin was fractionated into three species by repeated GPC, and their molar-based distribution of unit chains was determined by F-GPC (Fig. 2). The three species showed almost similar, bimodal distribution with the peak dp of 42–48 and 12–14, resembling those of their parent amylopectin. Their cl_n (19–21) was also similar. The C-chain distribution was examined after isoamylolysis of labeled amylopectin species, where only C chains were labeled. Again, the distribution of all the species resembled each other and was similar to that of their parent amylopectin (Fig. 3), although the amount of C chains by mole was smaller for larger species because the same amount by weight was loaded on the column. These results implied that all three species were basically

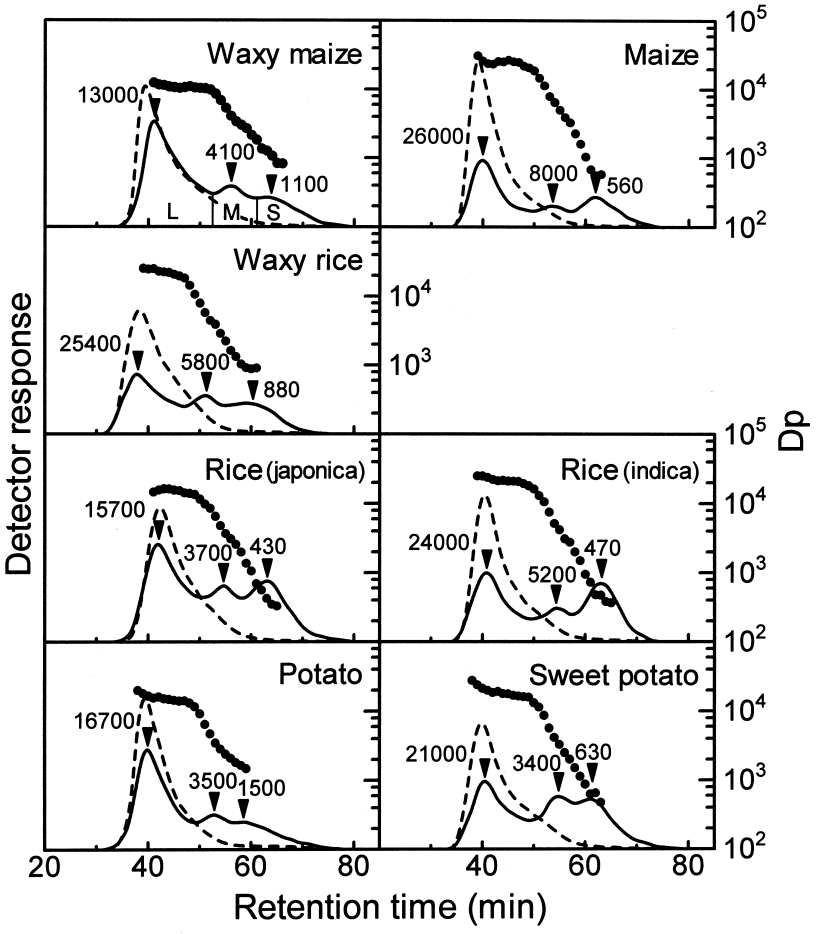

Fig. 1. Molar- and weight-based distributions of amylopectins from various plant sources. Solid line, molar-basd distribution by a fluorescent detector; dash, weight-based distribution by a refraction index detector; filled circle and number with arrowhead, dp; L, M and S, fractions of the large, medium and small species, respectively.

Table 1
Dp_n of amylopectin and its molecular species, and the proportion of the species

	Maize		Rice			Sweet potato	Potato
	Waxy	Normal	Waxy	Japonica	Indica		
Amylopectin							
Dp_n	9600	15,900	12,900	8200	10,900	9900	11,200
Species							
Dp_n							
Large	13,400	26,500	22,000	14,300	21,200	19,200	16,100
Medium	4400	8400	7200	5100	6700	5400	5500
Small	1500	1100	1200	700	800	900	2100
Proportion by mole (%)							
Large	63	54	49	49	45	43	61
Medium	18	16	28	20	17	25	18
Small	19	30	23	31	38	32	21
Proportion by weight (%)							
Large	88	90	83	85	86	84	88
Medium	9	8	15	12	11	13	8
Small	1	2	2	3	3	3	4

composed of a similar cluster-unit and differed in number of clusters per molecule. The dp_n of the large, medium and small species were 19,000, 6100 and 1900, respectively. The values were close to those obtained from the chromatogram of whole amylopectin (Table 1).

Each amylopectin cluster was suggested to comprise of 10.0–10.8 chains on average for rice and normal maize, 8.9–9.5 chains for sweet potato and 5.4–6.5 chains for potato.[4] From these values together with cl_n (18–23) and dp_n of the species, calculation of the number of clusters per molecule of each species should be possible by the equation, $(dp_n)/[(cl_n) \times (\text{number of chains per cluster})]$ (Table 2). The number of clusters per molecule differed by plant origin and appeared to be in range of 61–120 on average for the large species, 20–40 for the medium species, and 4–15 for the small species.

Gallant et al.[6] recently suggested the presence of blocklets organizing starch granule, based on observation under scanning and transmission electron microscopes. The blocklet was spherical, and large and small blocklets could be present. The diameter of a blocklet was 20–500 nm, being different by plant source. The blocklet was built up with several layers of crystalline lamellae, which was about 10 nm thick. Since a single cluster is 9–10 nm long, which is a similar size as for crystalline lamella, the diameter of the blocklet corresponded to be 2–50 cluster units in length arranged in tandem. Therefore, the number of clusters per molecule (Table 2) might imply that the molecule of the large

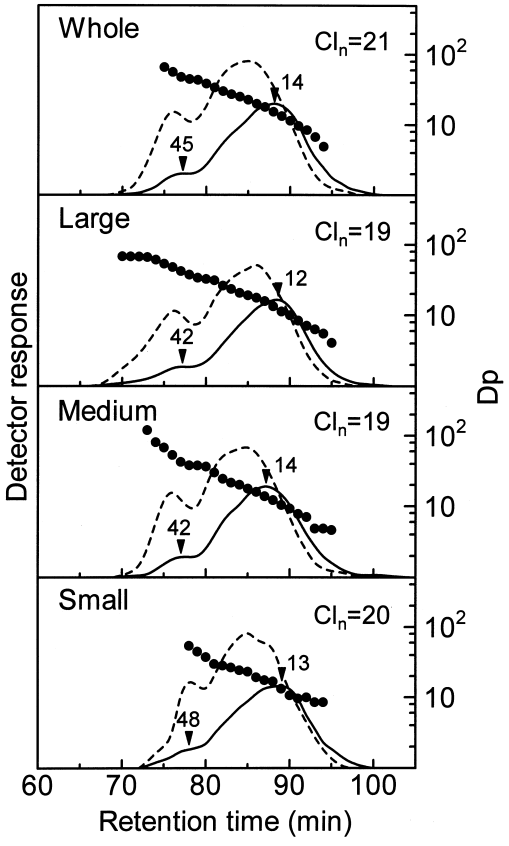

Fig. 2. Molar-based distribution of unit chains of waxy rice amylopectin (whole) and fractionated amylopectin species. Solid line, molar-based distribution; dash, weight-based distribution; filled circle and number with arrowhead, dp.

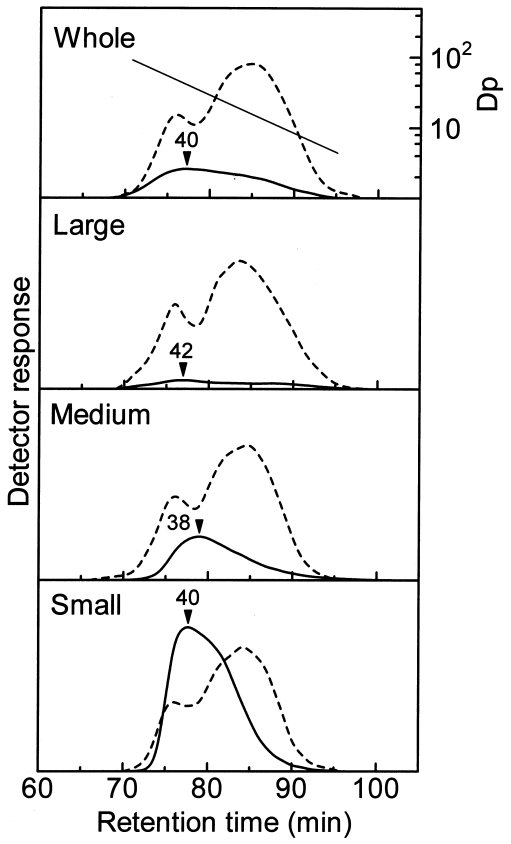

Fig. 3. Molar-based distribution of C chains of waxy rice amylopectin (whole) and fractionated amylopectin species. Solid line, molar-based distribution; dash, weight-based distribution of whole unit chains; number with arrowhead, dp. Dp was determined using the calibration line (the top panel), which was obtained by analyses of unit-chain distribution (Ref. 4).

and medium species itself was a blocklet. It was also possible that several molecules of these species formed a blocklet together, especially a fairly large blocklet. Some of the medium species, as well as the small species, was probably immature molecules or degraded products of the large species. The origin of these species would be clarified by following the changes in amount of each species during development or germination.

Average molecular-weight (average dp) of amylopectin has been reported by many investigators. Light scattering techniques, which in combination with other appropriate methods give weight-average molecular weight (M_w), has been most frequently applied. Most of the reported M_w values fall in a range of $0.1–13.9 \times 10^8$ (equivalent to weight-average dp (dp_w) $0.06–8.6 \times 10^6$; Ref. 13, 14). Number-averaged value for the macromolecules' size can be estimated by determination of reducing residues by a modified Park–Johnson method.[8] Hizukuri and co-workers reported dp_n of amylopectins to be from 0.47×10^4 (Ref. 11) to 1.5×10^4 (Ref. 15), which is reasonably close to the values obtained in this study. The difference by two orders of magnitude between dp_w and dp_n indicates very wide size-distribution of amylopectin molecules. With its capability of direct dp_n determination, F-GPC seems to be helpful for better understanding of molecular structure and biosynthesis/degradation process of amylopectin and amylose.

1. Experimental

1.1. Materials

Amylopectins were fractionated from defatted starch as described previously.[12] Starches used were prepared in the laboratory from waxy rice (cv. Medzurumochi), japonica rice (cv. Sasanishiki), indica rice (cv. IR42), potato (cv. Eniwa) and sweet potato (cv. Koganesengan), and waxy (white dent) and normal (dent) maize starches were commercial products (Sanwa Denpun Co., Nara, Japan). *Pseudomonas* isoamylase was purchased from Hayashibara Biochemical Lab. Inc. (Okayama, Japan). Other reagents were of the highest grade commercially available.

1.2. F-GPC

The labeling of amylopectin with 2-aminopyridine followed by GPC was performed by the method[3] with

Table 2
Number of clusters per amylopectin molecule of each species [a]

Species	Maize		Rice			Sweet potato	Potato
	Waxy	Normal	Waxy	Japonica	Indica		
Large	61	120	116	75	111	103	117
Medium	20	38	38	27	35	29	40
Small	6.8	5.0	6.3	3.7	4.2	4.8	15

[a] Calculated by $(dp_n)/[(cl_n) \times (\text{number of chains per cluster})]$, where the values of cl_n and number of chains per cluster (nc) used were from Ref. 4, 11. The nc for waxy maize and three cutivars of rice was assumed to be those of normal maize (10.0) and rice cv. Nihonbare (10.1), respectively.

modifications. A GPC system consisted of a degasser (Erma), a HPLC pump (JASCO PV-1580), a line filter (10 μm), a packed column (see below), and fluorescent (JASCO FP-920, excitation 315 nm, emission 400 nm) and refractive index (Erma ERC-7513) detectors connected in order. The column (Pharmacia HR 10/30) was packed with Toyopearl HW-40S, HW-50S and HW-75S (three layers, 4, 12 and 8 mL each in order), and maintained at 37 °C. The eluent was 50% Me_2SO–50 mM NaCl and the flow rate was 0.2 mL/min. The labeled amylopectin (5 mg), precipitated by EtOH (final conc. 75%) after labeling followed by washing with 75% EtOH–50 mM NaCl by centrifugation, was dissolved in Me_2SO (500 μL) by heating in a boiling water bath and was added water (500 μL). The resulting solution was clear. After filtration with membrane filter (10 μm, Millipore), an aliquot (100 μL) of the filtrate was loaded. The chain-length distribution of amylopectin and the C-chain distribution were determined by the method of Hanashiro at al.[4] Dp_n of amylopectin and its molecular species was calculated from the equation, $[(RI/F)_{sample}/(RI/F)_{standard}] \times (dp_n)_{standard}$,[3,4] where RI and F were the response of refractive index and fluorescent detectors, respectively.

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