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Note

Structure and physicochemical properties of starches from kidney bean seeds at immature, premature and mature stages of development

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Abstract

Starches from kidney bean (*Phaseolus vulgaris* L. cv. Toramame) seeds at the immature, premature, mature stages of development were examined. The starch content increased from 94, 219 to 265 mg per seed. Starches showed the C_a-crystalline type composed of small (< 5 μm) and large (10–35 μm) granules, with the large granules largely increasing with maturity. The amylose content increased from 21, 26 to 27%, and rapid viscograms and DSC thermograms suggested that the mature-stage starch was gelatinized with ease. The amylose increased in size from DPn 820, 1000 to 1080 and a number of chains per molecule (NC) from 3.3, 4.2 to 4.5. The branched amylose was a minor component (11–18% by mole) with NC 20–22. The amylopectin was similar in CL (23), β-amylolysis limit (59%), and chain-length distribution, but reduced in size (DPn 17,100–5270) and increased in content of phosphorus (114–174 ppm) with an increase in the amount of phosphorus linked to C-6 of the glucose residue (8–66%).

Keywords: Starch; Kidney bean; Structure; Physicochemical property; Seed development

Amylose and amylopectin are the main α-glucan components of starch. Amylose is small, linear or slightly branched molecules whereas amylopectin is large, highly branched molecules. The biosynthesis of these glucans is a complex, concerted process in which several classes of enzymes, starch synthase, branching enzyme and debranching enzyme, are involved. Although the specific role of the respective enzyme in determining the molecular structure has been proposed in the literature,[1,2] there are still many aspects to be clarified. In contrast to the number of studies on the biosynthetic enzymes, only a few investigations on structural changes of the starch itself during the maturation of starch-storing tissues have been carried out.[3–5] Parallel knowledge on both the properties of enzymes and the structures of their products is expected to help better understanding of the starch biosynthesis. In this study, the starches were prepared from kidney bean at different developmental stages and their molecular structure and some physicochemical properties were examined in detail.

The starch amounts of the seeds were 94, 219 and 265 mg per seed for immature, premature and mature stages, respectively, which corresponded to 37, 43 and 38% by dry weight. Starch granules were oval, similar to potato starch granules (Fig. 1). The starches were composed of small (< 5 μm) and large (10–35 μm) granules similar to wheat and barley starches. The proportion of large granules, especially > 25 μm, increased from the immature to premature stage (Fig. 2).

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As reported for barley starch,[6] the structural analysis of isolated small and large granules will clarify whether small granules were enlarged or large granules were produced independently.

The actual contents of amylose were 21, 26 and 27% for the immature, premature and mature stages, respectively. A relatively large increase in the content (5%) was found from the immature to premature stage (Table 1). The apparent amylose contents were 4–5% higher than the actual content because of a relatively high iodine-affinity (IA) of amylopectins, suggesting that the amylopectins had very long chains (LC chain).[7] The amount of amylose–lipid complex was as small as 1–9% against total amylose, corresponding to only 0.2–2% of starch. SDS-PAGE of waxy protein (granule bound starch synthase I, GBSS I) is shown in Fig. 3. One major band was seen for all the starches, and N-terminal amino acid sequence of the major 58 kDa-protein was almost identical to that of rice waxy protein (Table 2). The amount of the protein increased during development, especially from the premature to immature stage, being consistent with the increase of amylose content.

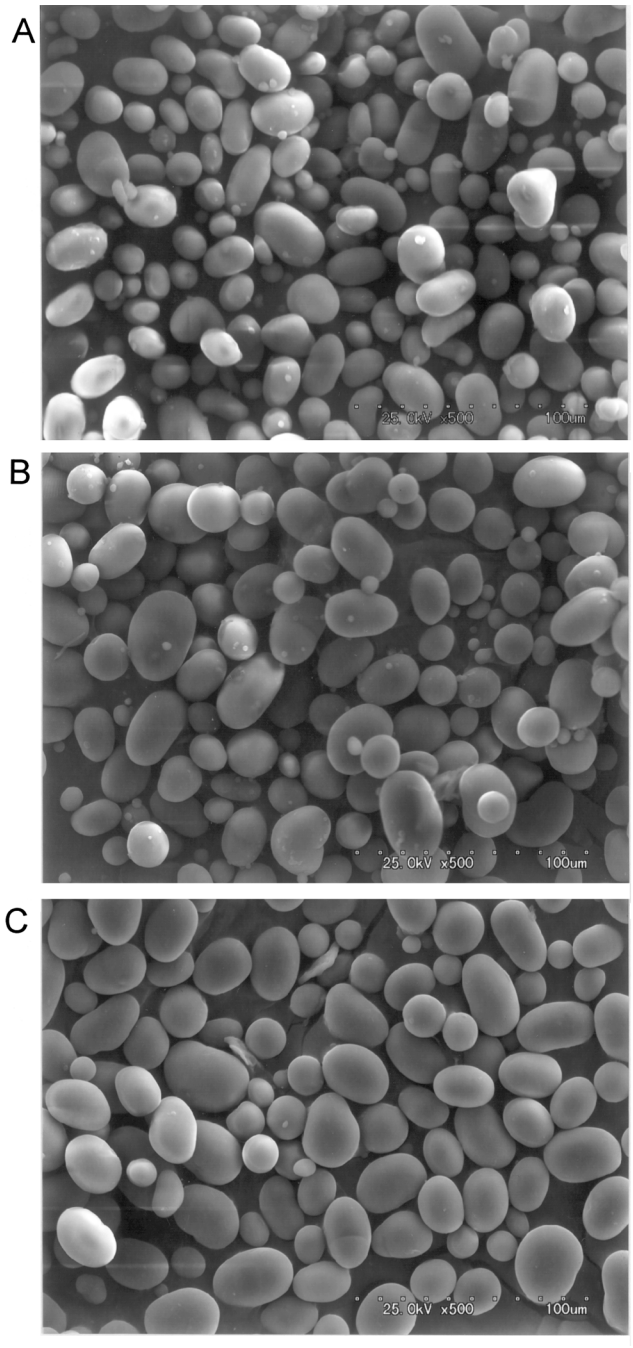

Fig. 1. Scanning electron micrographs of kidney bean starches. A, B and C are the granules at the immature, premature and mature stages, respectively.

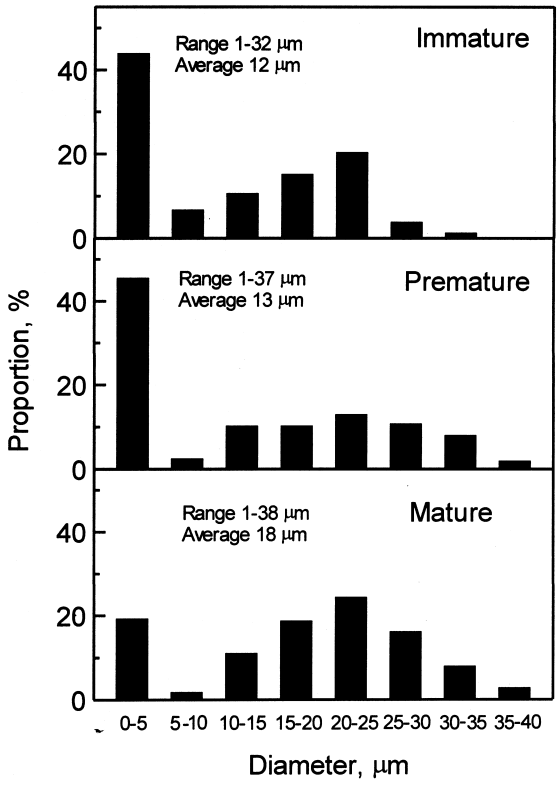

Fig. 2. Granule size distribution of kidney bean starches. The proportions by number were from scanning electron micrographs.

Starches of all the stages showed the C_a-crystalline type (data not shown), being the same as those of developing smooth peas and intermediate between other legumes (C-type) and mung bean (A-type).[8] Rapid viscograms of starches showed no apparent peak and breakdown (Fig. 4). The premature/mature-stage starches had a higher viscosity at 40 °C on cooling than the immature-stage starch, indicating that the former starches had a higher retrogradation tendency because of their higher amylose contents. The thermal properties (Table 1) of starches varied with seed development. The decrease in ΔH agreed with the increase of amylose content. Similar thermograms (not shown) were ob-

Table 1
Iodine affinity (IA), amylose content and endothermic properties of kidney bean starches

Stage	Iodine affinity (g/100 g) of starch				Amylose content [a] (%)	Endothermic characteristics [b]			
	Defatted (A)	Non-defatted (B)	A − B	(A − B)/A		T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Immature	5.07	4.62	0.45	0.09	21 (25) [c]	65.0	69.8	90.7	17.2
Premature	6.11	6.05	0.06	0.01	26 (31)	66.9	70.5	94.3	15.5
Mature	6.38	6.31	0.07	0.01	27 (32)	61.5	66.8	90.8	15.4

[a] Calculated from $[(IA_{defatted\ starch} - IA_{amylopectin})/(IA_{amylose} - IA_{amylopectin})] \times 100$. For IA of amylose and amylopectin, see Tables 3 and 4, respectively.
[b] Values are the average of three separated determinations. T_o, T_p and T_c are onset, peak and completion temperatures, respectively.
[c] Apparent content calculated from $[(IA_{defatted\ starch})/20] \times 100$.

tained for the starches, i.e., a peak with a shoulder, which suggested the presence of two types of crystallite. The absence of an endothermic peak around 100 °C suggested that no amylose–lipid complexes were present as for other legume starches[9] and consistent with a small increase in IA by defatting (Table 1). These results showed that RVA and DSC characteristics changed during the seed development and the mature-stage starch was gelatinized more easily than the other-stage starches. No such change was observed for normal maize kernel during development.[10]

The properties of amyloses are summarized in Table 3. The immature-stage amylose had a smaller number-average DP (DPn) than the others, although the weight-average DP (DPw) was similar. The amyloses were similar in size to rice and maize amyloses, but smaller than root and tuber amyloses.[11] The average chain-length (CL) and β-amylolysis limit were similar in all the stages, but the immature-stage amylose showed a smaller number of chains per molecule than the others. Figure 5 shows that amyloses were comprised of three components, large (L), medium (M) and small (S) as in the case for water chestnut,[12] sago,[13] iburu, acha,[14] sweet potato and potato.[15] The weight proportion of the M-component increased with seed development, in agreement with increase of DPn.

β-Limit dextrin (β-LD) of amylose was prepared for characterization of the branched molecules (Table 3). β-LDs were similar in IA, blue value and λmax, and these values were lower than those of the respective amyloses. β-LD had a larger DPn than the parent amylose, suggesting that the branched molecules were larger than the linear molecules as reported previously for other plant amyloses.[11,16] From a comparison of the chromatograms between amyloses and β-LDs (Fig. 5), it was suggested that L- and M-components were branched amyloses while the S-component was mainly linear amylose. The CL of β-LDs slightly increased from 42 to 58 during the development, but the average number of chains per molecule (NC) was similar. The CL was lower and the NC was higher than those of

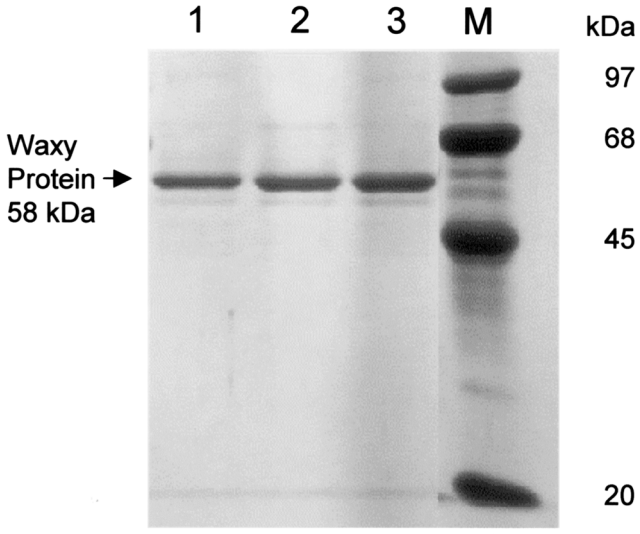

Fig. 3. SDS-PAGE of waxy protein. Lane 1, 2 and 3 are extracts from starches of the immature, premature and mature stages, respectively.

Table 2
N-Terminal amino acid sequence of waxy protein of kidney bean [a]

Kidney bean	1 GMNLIFVGAEVAPWSKTGGLGDVLGGLPSA 30
Rice[b]	1 ATGAGMNVVFVGAEMAPWSKTGGLGDVLGGLPPA 34

[a] Shaded letters indicate identical amino acid residues between the two sequences.
[b] From Ref. 36.

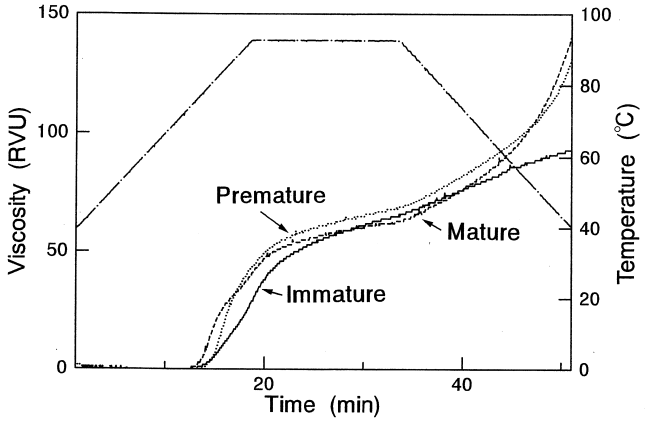

Fig. 4. Rapid viscograms of kidney bean starches (9%).

cereals, roots and tubers (CL 63–160, NC 5.3–17.1, Ref. 11). NC of branched molecules implied that the branched structure was similar throughout seed development. The most significant change in amylose structure was an increase in molar proportion of branched molecules, although the proportion was much smaller than those of other plants (27–70%).[11]

Table 4 shows the properties of amylopectins. The IA, blue value and λmax increased slightly on the seed development. The high IA was similar to that of maize (1.10–1.25).[17] The CL was comparable to those of potato (23) and maize (22). The constant β-amylolysis limit (59%), together with the CL values, implied the same branching pattern of unit chains, and probably the same structure at a cluster level, which was maintained throughout the developmental stage of the seeds. The most remarkable difference was large decrease in

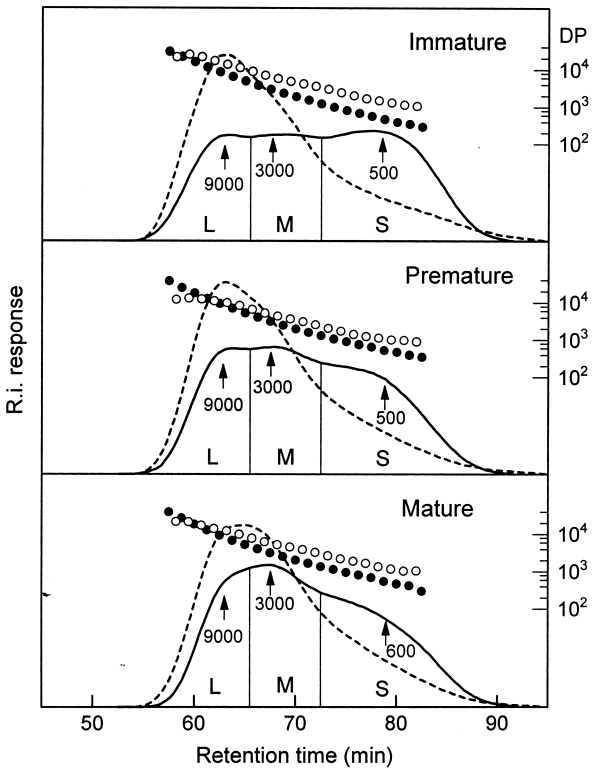

Fig. 5. Gel-permeation HPLC chromatograms of kidney bean amyloses and their β-limit dextrins. — and ···: amylose and β-limit dextrin, respectively. ● and ○: DP of amylose and β-limit dextrin, respectively.

DPn, as confirmed by GPC (Fig. 6) and by decrease of the limiting viscosity number.

Chain-length distributions of amylopectins were determined by gel-permeation high performance liquid

Table 3
Properties of kidney bean amyloses

Property	Immature		Premature		Mature	
	Amylose	β-LD	Amylose	β-LD	Amylose	β-LD
Iodine affinity, IA (g/100 g)	20.0	—[a]	19.9	16.3	19.6	16.3
Blue value	1.46	1.08	1.47	1.18	1.49	1.20
λmax (nm)	652	636	653	642	653	642
Number average DP, DPn	820	920	1000	1190	1080	1170
Weight average DP, DPw	4100	10,100	4500	6600	4100	6100
Apparent DPw distribution	200–20,000	2000–39,500	200–20,000	2000–21,800	200–18,000	2000–29,000
Average chain-length, CL	250	42	240	55	240	58
Number of chains per molecule, NC	3.3	22.0	4.2	21.8	4.5	20.1
β-Amylolysis limit, β-AL (%)	92		91		90	
Proportion (% by mole) [b]						
Linear amylose	89		85		82	
Branched amylose	11		15		18	

[a] Not determined.
[b] Calculated from $[(NC_{amylose}-1)/(NC_{\beta\text{-LD}}-1)]\times 100$.

chromatography (HPLC) with LALLS and HPAEC-PAD (Fig. 7). There was no significant difference in the distributions with seed development. Amylopectins showed (panel A) a shoulder (DP 200–220) and two peak summits (DP 40–44 and 16–18). The chains were fractionated into very long chain (LC), B4–B1 and A in order of elution.[18] Each fraction of the respective stage showed similar carbohydrate amount and CLw (Table 5), but the amount of long chain fractions (LC–B3) increased slightly from 10 to 12% concomitantly with the slight increase of IA, blue value and λmax. The long chains might be produced by GBSS I,[19] and the increase of long chains agreed with the increased amount of the waxy protein as mentioned above. The CLw of the fractions was similar to those of wheat and barley,[18] but the amount of A fraction was much smaller and, conversely, that of B1 fraction was much larger.[6,20,21] By HPAEC-PAD (panel B) the highest peak chain was observed at DP 13. The chains of DP 6–8 decreased in amount in order and those of DP >9 increased. This characteristic DP distribution was observed for potato and sweet potato but not for cereals.[22] These findings suggested that branching enzymes of kidney bean was similar to those of potato and sweet potato in short-chain transfer.

In this study, changes in physicochemical properties and molecular structure of kidney bean starch with seed development have been investigated. As the seeds matured, the proportion of larger granules and amylose content increased. Presumably these changes caused differences in pasting and thermal properties. The only structural change in amylopectin was a decrease in DPn, suggesting that, as long as clusters with normal structure are formed, granular formation could occur

Table 4
Properties of kidney bean amylopectins

Property	Immature	Premature	Mature
IA (g/100 g)	1.22	1.25	1.47
Blue value	0.181	0.185	0.188
λmax (nm)	561	562	564
CL			
Smith degradation	23	23	23
Isoamylolysis	23	23	22
β-AL (%)	59	59	59
DPn	17,100	10,500	5270
Limiting viscosity number (mL/g)	162	162	150
Phosphorus (ppm)			
Organic	114	174	174
Linking to C-6 of the glucosyl residue, P-6	9	59	115
P-6/organic (%)	8	34	66

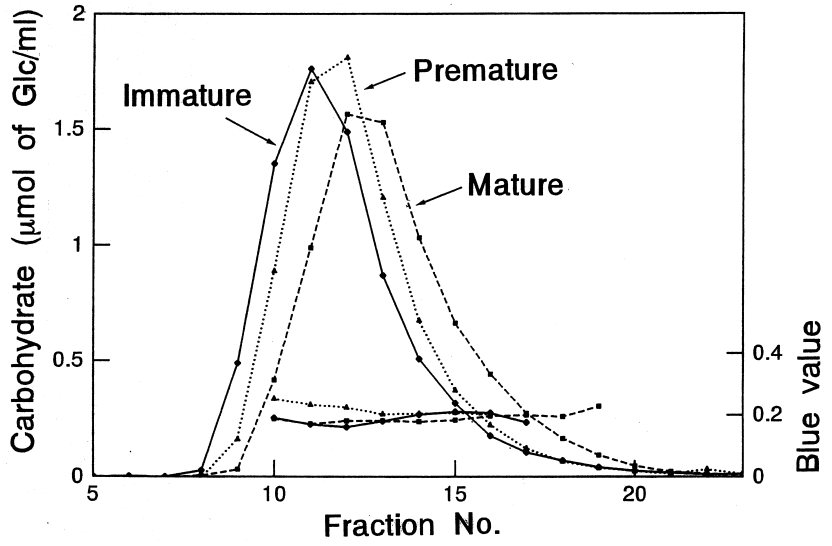

Fig. 6. Gel-permeation chromatograms of amylopectin on Toyopearl HW-75F.

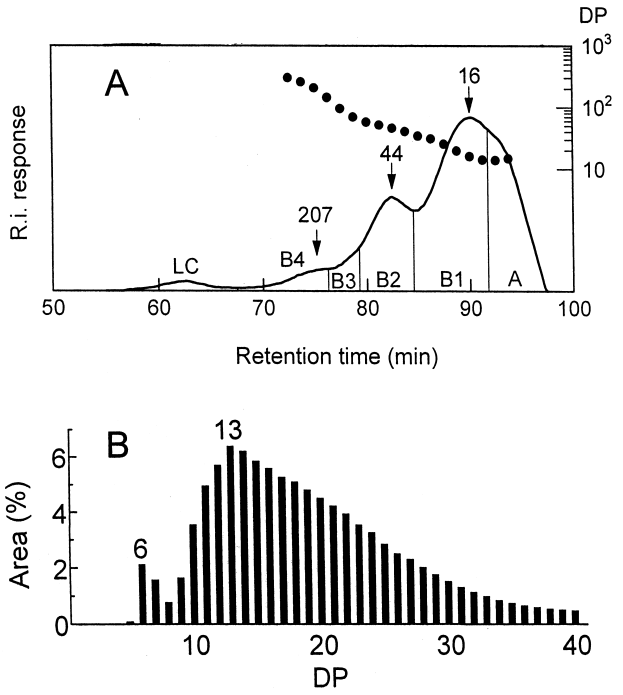

Fig. 7. Chain-length distribution of amylopectin from mature seeds determined by gel-permeation HPLC (A) and HPAEC-PAD (B). In panel A, ● and numbers with arrow indicate DP.

regardless of the size of amylopectin molecules. Structural change in amylose, both linear and branched molecules, was also subtle during seed development except for an increase in DPn and molar ratio of branched molecules.

1. Experimental

1.1. Materials

Kidney bean plants were grown in a field of Faculty of Agriculture, Hokkaido University. The seeds were harvested at three different stages of development and stored at −80 °C until use. Starch was isolated from the seeds by an alkaline steeping method.[17,23] Defatted starches were prepared by dissolution in Me_2SO and precipitation with EtOH.[24] The fractionation of amylose and amylopectin was performed by the method of Lansky et al.[25] with modifications.[24] The purity of amylose specimens was estimated by gel permeation chromatography on Toyopearl HW-75F.[24] β-Limit dextrin of amylose was prepared by the method of Takeda et al.[11] Sweet potato β-amylase from Sigma Chemical Co. was further purified by the method of Marshall and Whelan.[26] *Pseudomonas* isoamylase was purchased from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). Other reagents were of the highest grade commercially available.

1.2. Analytical methods

The starch content of seeds was determined by glucoamylase digestion after treatment with hot 50% EtOH. IA was determined at 25 °C by automated amperometric titration.[7] The blue value, λmax, limiting viscosity number (mL/g, 1 M KOH, 22.5 °C), DPn,[27] DPw,[28] CL,[27,29] and β-amylolysis limit[30] were determined as described previously. NC was DPn/CL. The chain distribution of amylopectin after isoamylolysis was determined by gel-permeation HPLC with a low-angle laser-light scattering photometer (LALLS)[18] and anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).[22] Carbohydrate was determined by the phenol–H_2SO_4 method.[31] Phosphorus content was determined as inorganic phosphate[32] after treatment of hot perchloric acid.[33] Phosphorus linking to C-6 of the glucosyl residue was determined with glucose-6-phosphate dehydrogenase after acid hydrolysis.[34]

X-ray diffraction was performed on wet specimens (Rotaflex RV-20013, Rigaku Denki Co., Tokyo, Japan) under the conditions described by Hizukuri et al.[12] Scanning electron micrographs were taken with a scanning electron microscope (S-2150, Hitachi, Ltd., Tokyo, Japan).[14] The diameters of starch granules were estimated by averaging the dimension of 100 granules that were randomly chosen from five micrographs. Pasting characteristics of 9% (w/w) starch suspensions were determined using a Rapid Visco-analyzer (RVA-3D, Newport Scientific Pty, Ltd., Narrabeen, Australia). Heating and cooling was at the rate of 3 °C/min. Dif-

Table 5
Carbohydrate amount and average chain-length (CLw) of fractions of isoamylase-debranched amylopectins

Amylopectin	Amount (% of total)						CLw					
	LC	B4	B3	B2	B1	A	LC	B4	B3	B2	B1	A
Immature	2	4	4	20	47	23	760	280	96	49	22	13
Premature	2	4	5	20	46	23	760	290	98	50	23	13
Mature	3	5	4	19	45	24	940	280	93	49	22	14

ferential scanning calorimetry was performed according to the method of Yoshimoto et al.[20] with the scanning temperature range of 25–150 °C.

Proteins were extracted from starch (20 mg) with water (125 μL) and 0.25 M Tris–acetate (pH 6.8) containing 5% SDS, 10% 2-mercaptoethanol and 20% glycerol (125 μL) by heating at 100 °C for 10 min. The extract was centrifuged at 14,000 rpm for 10 min and an aliquot of the supernatant was analyzed by SDS-PAGE with 10% separating gel.[35] After SDS-PAGE proteins were stained with Coomassie Brilliant Blue. N-Terminal amino acid sequence was determined with a gas-phase amino acid sequencer (Applied Biosystems Inc., CA, USA) after SDS-PAGE followed by blotting the separated proteins onto PVDF membrane (Millipore).

The size of amylopectin molecules was compared by means of GPC with Toyopearl HW-75F. Amylopectin (2.4 mg in 200 μL) was loaded onto a column (1.5 × 24 cm) and the chromatography was performed at 40 °C using 50 mM NaCl as eluent. Each fraction (2 mL) was analyzed for total carbohydrate and blue value as described above.

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