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The structure of the hot-water soluble components in the starch granules of new Japanese rice cultivars

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Abstract

In this study, the structures of the hot-water (80°C) soluble starch fractions (HWS) of six new Japanese rice cultivars (Saikai 194, Saikai 198, Hokuriku 149, Suigen 258, Hoshiyutaka, and Saikai 184) were investigated following a previous study [Mizukami, H., Hizukuri, S. and Takeda, Y. Structures and pasting properties of starches from new characteristic rice cultivars, Oyo Toshitu Kagaku (J. Appl. Glycosci.) 43 (1996) 15–23]. The HWS were subfractionated into 1-butanol-precipitate (SAM) and supernatant (SAP) fractions. The yields of the SAM and SAP fractions were 0.3%–2.4% and 3.1%–4.1% by starch weight, respectively. The Hoshiyutaka and the Saikai 184 yielded both relatively large (2.4%) and small amounts (0.3%) of SAM. The SAM were small amylose molecules with a $\overline{DP}_n$ between 320 and 420 and a $\overline{DP}_w$ between 950 and 1850. The SAM from the Hoshiyutaka and the Saikai 184 were the larger molecules with $\overline{DP}_n$ 390 and 420, respectively, and having slightly more branches (6.0 and 8.1) than those from the others (1.5–4.5). The SAP were smaller molecules having a $\overline{DP}_n$ between 60 and 190 as compared to the SAM. The SAP was composed of small amylopectin molecules ($\overline{DP}_n$ 280–790, $\overline{CL}$ 17–32, β-amylolysis limit (β-AL), 54%–68%) including very small amylose molecules ($\overline{DP}_n$ 24–34) having an average number of branch linkage ($\overline{NBL}$) of between 0.4 and 0.5. Both the amount and the structures of hot-water-extractable rice starch fractions vary with cultivar, and may influence their cooking properties.

Keywords: Rice cultivars; Hot-water-extractable fractions; Japanese rice; Starch

1. Introduction

Starch granules are heterogeneous mixtures of two kinds of molecule, amylose and amylopectin, which differ in molecular size and degree of branching. Amylose is a smaller component and an essentially linear α-1,4-glucan which contains only tiny amounts of α-1,6-branch linkages, while amylopectin is a larger component and a branched α-1,4- and α-1,6-glucan (Hizukuri, 1996; Manners, 1985; Morrison & Karkalas, 1990). The structure of starch can generally be determined when amylose and amylopectin are separated and purified using a completely dispersed aqueous solution containing helical complexing agent(s) such as 1-butanol, pentanol (Lansky et al., 1949; Takeda et al., 1986) and thymol (Haworth & Sagrott, 1946). The crystalline precipitate and the supernatant from the dispersed solution are amylose and amylopectin, respectively. The structures of amylose and amylopectin are characteristic of the botanical source (Hizukuri, 1996) and those of some rice starches were investigated in detail (Hizukuri et al., 1983; Takeda et al., 1986, Takeda et al., 1987a, Takeda et al., 1989a, Takeda et al., 1989b, Takeda et al., 1993; Villareal, et al., 1997). An alternative fractionation process is leaching the starch granules with hot water (Banks & Greenwood, 1975). Although the leached fraction is only a small portion of the total polysaccharide, this may provide basic information on the structure and function of the starch granule. The leached fraction may be further subfractionated with 1-butanol into a precipitate fraction (complex with 1-butanol) and a supernatant fraction (amylopectin). The precipitate fraction contains mostly small amylose molecules obtained from completely dispersed starch. The fraction is reported to be comprised of linear and slightly branched molecules (Banks et al., 1959, Cowie & Greenwood, 1957; Hizukuri, 1991; Schoch, 1945). The supernatant fraction is amylopectin (Cowie & Greenwood, 1957) but their CL-distributions were reported to be quite different from those of normal amylopectin (Murugesan et al., 1993).

The cooking properties of popular Japanese rice varieties are quite similar to numerous other rice varieties found

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Table 1
Origins and properties of rice grains and starches.[a]

Cultivar	Parents (Mother × Father)	Grain property and yield	Apparent amylose content (%)	Maximum viscosity (RVU, 10%)	Breakdown (%)
Saikai 194	Hokuriku 127[b] × SLG[d] 9	Large grain,high yield	19.1	310	39
Saikai 198	Suigen 258[e] × Hokuriku 133[f]	Slightly long grain, high yield	18.5	348	51
Hokuriku 149	Mitsuyou 23[c] × Akihikari[b]	Thin and long grain	19.0	349	48
Suigen 258	Unknown, from Korea	Small grain, very high yield	21.0	332	42
Hoshiyutaka	Chugoku 55[b] × KC[g] 89	Long grain, less sticky	27.5	231	25
Saikai 184	Suigen 258[e] × IR2061	Long grain, high yield	30.1	371	8

[a] Mizukami et al., 1996.
[b] Japonica.
[c] From Korea (having ancestoer of indica).
[d] Super large grain.
[e] Has an indica ancestor.
[f] Has parents from Korea.
[g] Initials of creator.

throughout the world. However, some new cultivars with different cooking properties have recently been created in Japan in order to find new uses for rice. These new cultivars have some advantages in terms of yield and have properties allowing diverse uses. In a previous paper (Mizukami et al., 1996), we reported on the molecular structures of whole amylose and amylopectin fractionated by complete dispersion, and on the pasting properties of starches within six new rice cultivars. Two of these starches, Hoshiyutaka and Saikai 184, showed structures and pasting properties distinguishable from the others. In this paper, we further investigate the structures of the hot-water-extractable fractions of these starches.

2. Materials and methods

2.1. Materials

Starches from the new characteristic rice cultivars, Saikai 194, Saikai 198, Hokuriku 149, Suigen 258, Hoshiyutaka and Saikai 184, were prepared by an alkaline steeping method (Yamamoto et al., 1973) from polished rice which were gifts from the Kumamoto Prefectural Agricultural Research Center. These rice starches were the same specimens as those used previously (Mizukami et al., 1996). Their genetic origins and some properties of the grains and starches are summarized in Table 1.

2.2. Preparation of the hot-water soluble fraction and its subfractionation

A starch suspension (40 g/3 L distilled water, pH 6.5–7.2) was kept at room temperature for 20 min and then heated at 80°C for 1 h while being with stirred under a nitrogen atmosphere. A hot-water soluble fraction (HWS) was separated by centrifugation from the suspension at 15 000 × *g* for 10 min. One-tenth the volume of 1-butanol was added to the HWS and the mixture was incubated at 30°C or 24 h. The resulting precipitate (amylose fraction, SAM) was collected by centrifugation at 15 000 × *g* for 10 min and washed with ethanol first and later with ether,

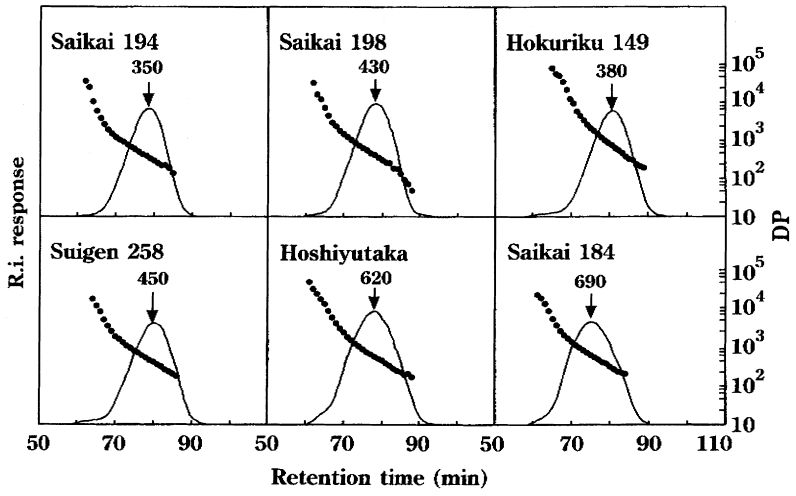

Fig. 1. Gel-permeation HPLC of SAM, (—) response of differential refractometer; (●) DP

Table 2
Yields (weight %) of hot-water soluble fractions (HWS) and their SAM and SAP.

Cultivar	HWS[a] (%)	SAM[a] (%)	SAP[a] (%)	SAM/AM[b](%)	SAP/AP[c] (%)
Saikai 194	4.5	0.7	3.8	4.1	4.5
Saikai 198	4.5	1.4	3.1	8.6	3.7
Hokuriku 149	4.3	0.5	3.8	3.0	4.5
Suigen 258	4.1	0.7	3.4	3.9	4.1
Hoshiyutaka	6.4	2.4	4.0	11.5	5.1
Saikai 184	4.4	0.3	4.1	1.6	5.0

[a] From starch granules.
[b] Amylose.
[c] Amylopectin.

and dried over calcium chloride under reduced pressure. The supernatant (amylopectin fraction, SAP) was concentrated by a rotary evaporator under reduced pressure and lyophilized. The SAP was further fractionated after being dissolved in 1 M of sodium hydroxide and neutralized with 1 M hydrochloric acid. The solution (10 mL) (Saikai 194: 12 mg/mL, Hoshiyutaka: 15 mg/mL, Saikai 184: 10 mg/mL) was applied to a column (2.6 × 100 cm) packed with a mixed gel of Toyopearl HW-55F and HW-65F (1 : 1) and eluted with 50 mM sodium chloride, at a flow rate of 0.25 mL/min and 35°C. The eluent was fractionated into three subfractions of large (SAP-L), medium (SAP-M) and small (SAP-S) molecules according to the elution chromatogram (Fig. 2). These subfractions were concentrated under reduced pressure and stored at − 30°C before use.

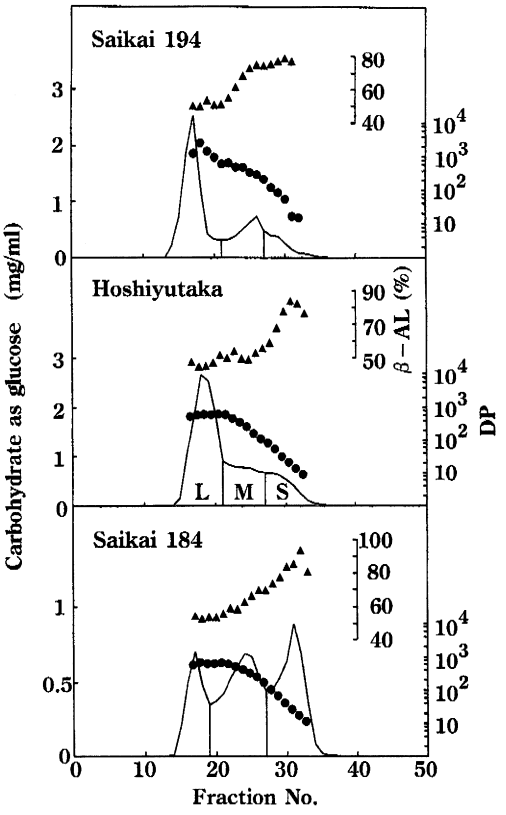

Fig. 2. Gel-permeation chromatograms of SAP on mixed gel of Toyopearl HW55-F and 65-F, carbohydrate; (●) $\overline{DP}_n$; (▲) β-amylolysis limit

2.3. Methods

Total carbohydrate and reducing terminal residues were determined by the phenol–sulfuric acid method (Dubois et al., 1956) and the modified Park–Johnson method (Hizukuri et al., 1983), respectively. The number-average degree of polymerization ($\overline{DP}_n$) was calculated by dividing total carbohydrates by the reducing residues. The number-average chain length ($\overline{CL}$) was determined by both the rapid Smith degradation method (Hizukuri et al., 1981) and hydrolysis with isoamylase (Suzuki et al., 1981). The β-amylolysis limit (β-AL), the blue value and the λ_{max} were measured by the methods of Suzuki et al., (1981).

The weight-average DP ($\overline{DP}_w$) and the DP distribution of the SAM were analyzed by gel-exclusion HPLC which was monitored with a low-angle laser-light scattering photometer (LALLS) and a differential refractometer (RI), as was reported by Murugesan et al. (1993). The chain length distribution of each subfraction of the SAP following debranching with isoamylase (Mizukarni et al., 1996; Suzuki et al., 1981) was also analyzed by HPLC–LALLS–RI (Hizukuri, 1986), and the conditions were modified as follows. The specimen (2.5–4.5 mg, 500 μl) was loaded on to three columns, connected in tandem, of GS-520, GS-320 (both 7.6 × 500 mm, Asahi Chem. Co.) and TSK-GEL G2000PW (7.5 × 600 mm, Toso) and eluted with 0.1 M of sodium phosphate buffer, pH 6.1 containing a 0.02% sodium azide and 1.5% dimethyl sulfoxide with a flow rate of 0.5 mL/min.

3. Results

3.1. Hot-water soluble fraction amounts of and their subfractions

Table 2 shows the yields (%, w/w) of the HWS, and its subfractions. The HWS yields were in the range of 4.1%–6.4%. Hoshiyutaka gave a slightly higher yield (6.4%) than

Table 3
Properties of SAM

Cultivar	$\overline{DP}_w$	$\overline{DP}_n$	$\overline{CL}$[a]	$\overline{NBL}$[b]	β-Al[c](%)	Blue value	λ_{max} (nm)
Saikai 194	970	320	200	0.6	87	0.98	628
Saikai 198	1070	330	170	0.9	88	0.97	626
Hokuriku 149	960	350	200	0.8	86	1.00	627
Suigen 258	1250	330	180	0.8	82	0.97	627
Hoshiyutaka	1850	390	110	2.5	66	0.96	627
Saikai 184	1530	420	140	2.0	72	0.99	634

[a] Average chain length.
[b] Average number of branch linkages.
[c] β-Amylolysis limit.

those (4.1%–4.5%) of the other cultivars. The yields of the SAP were similar (3.1%–4.1%), but those of SAM differed with cultivar (0.3%–2.4%). Saikai 184 released a very small amount of the SAM (0.3%), whereas Hoshiyutaka leached a relatively large amount (2.4%). SAM was only a minor component in these starches, but the proportions to whole amyloses were as high as 11.5% and 8.6%, for Hoshiyutaka and Saikai 198, respectively, and much less (1.6%–4.1%) for the other cultivars. Thus, the amounts of SAM varied widely between cultivars.

3.2. The structure of the SAM

The SAM from all cultivars showed similar elution profiles with a single peak on gel-permeation HPLC (Fig. 1), but the degree of polymerization ($\overline{DP}$), at the peaks and DP distribution, varied for the cultivars. Hoshiyutaka and Saikai 184 had peaks at DP 620 and 690, respectively, which were considerably larger than the other cultivars (which had a peak DP, between 350 and 450). The apparent DP distributions, which were indicated by a ($\overline{DP}_w$) 10% of the highest and the lowest molecular weight fractions (Hizukuri, 1985), were 250–2420 and 250–2190 for Hoshiyutaka and Saikai 184; respectively, being a wider range than those of the others (Saikai 198, 230–1140, Saikai 194, 210–940; Hokuriku 149, 210–1180; suigen 258, 200–1230). The large molecular fraction of SAM seemed to be more highly branched than the small molecular fraction, judging from the steep slope of the DP plot lines.

The weight- and the number-average DP ($\overline{DP}_w$ and $\overline{DP}_n$, respectively) were in the ranges of 960–1850 and 320–420, respectively (Table 3), indicating that the SAM had much smaller molecules (about one-third in size) than those of the whole amyloses ($\overline{DP}_w$, 3200–3830; $\overline{DP}_n$, 860–1150) obtained by the dispersion method (Mizukami et al., 1996). The SAM of Hoshiyutaka and Saikai 184 had larger molecules than those of others. The average $\overline{CL}$ ranged from 110 to 200, with Hoshiyutaka showing the smallest value, and Saikai 194 and Hokuriku 149 showing the largest value (Table 3). The average number of branch linkages ($\overline{NBL}$) and the β-AL of the SAM were in the range of 0.6–2.5 and 66%–88%, respectively. The SAM with the lower $\overline{NBL}$ had a higher β-AL, as in the case of the whole amyloses reported on rice amyloses and other sources (Hizukuri, 1996; Hizukuri et al., 1989). The SAM had lower $\overline{NBL}$ values than those of whole amyloses (1.0–4.5) (Mizukami et al., 1996). Saikai 194, Saikai 198, Hokuriku 149 and Suigen 258 had $\overline{NBL}$ values of less than one (0.6–0.9), indicating that these SAM were composed of linear and branched molecules as shown in amyloses from various sources (Hizukuri, 1996; Takeda et al., 1987b). The blue values (0.96–1.00) were 31%–39% lower and the λ_{max} were 21–27 nm shorter than those of the whole amyloses previously reported (Mizukami et al., 1996), as the molecules of the SAM are smaller.

3.3. Structure of SAP

The $\overline{DP}_n$ values of the SAP were in the range of 60–190. Saikai 194 had the largest molecule while Saikai 184 had

Table 4
Properties of SAP

Cultivar	$\overline{DP}_n$	$\overline{CL}$ Smith[a]	Iso-A[b]	$\overline{NBL}$	β-AL (%)	Blue value	λ_{max} (nm)
Saikai 194	190	21	20	8.1	52	0.17	564
Saikai 198	120	22	21	4.5	54	0.23	571
Hokuriku 149	130	22	22	5.0	52	0.20	564
Suigen 258	130	21	22	5.1	54	0.19	564
Hoshiyutaka	150	21	21	6.0	52	0.18	562
Saikai 184	60	24	24	1.5	63	0.24	558

[a] Determined by the rapid Smith-degradation method.
[b] Determined by hydrolysis with isoamylase.

Table 5
Properties of subfractions of SAP

Cultivar	Subfraction	Proportion (weight %)	$\overline{DP}_n$	$\overline{CL}$[a]	$\overline{NBL}$	β-AL (%)
Saikai 194	L	66 (2.5)[b]	790	18	43	55
	M	24 (0.9)	350	32	9.9	68
	S	10 (0.4)	34	23	0.5	76
Hoshiyutaka	L	60 (2.4)	470	17	27	54
	M	26 (1.0)	280	18	15	60
	S	14 (0.6)	29	20	0.5	84
Saikai 184	L	21 (0.9)	490	17	28	56
	M	43 (1.8)	430	29	14	59
	S	36 (1.5)	24	17	0.4	85

[a] Determined by the rapid Smith-degradation method.
[b] Ratio of subfraction to whole starch.

the smallest molecule among the cultivars (Table 4). All the SAP were smaller than the SAM, the reverse order is seen in normal amylose and amylopectin, is therefore much smaller than the whole amylopectin ($\overline{DP}_n$; 6900–10 700) (Mizukami et al., 1996). The $\overline{CL}$ of the SAP was in the range of 20–24, which is 10%–20% longer than that of normal rice amylopectin. Both $\overline{CL}$ values determined by rapid Smith degradation and hydrolysis with isoamylase coincided suggesting that all the branches were made by α-1,6-linkages. The SAP of Saikai 184 had the largest CL, being two or three glucose residues longer than those of the others. The SAP showed an $\overline{NBL}$ of 1.5–8.1, and Saikai 184 and Saikai 194 had the lowest and highest $\overline{NBL}$, respectively. The others were in a similar range (4.5–6.0). The β-AL of Saikai 184 was 63% being about 10% higher than those (52%–54%) of the others, which were in a slightly lower range than previously reported for whole amylopectins (55%–57%, Mizukami et al., 1996). The blue values were in the range 0.17–0.24. These values were much higher than those (0.06–0.08) of the whole amylopectin with the exception of Hoshiyutaka (0.16) of which was similar (0.18) but Saikai 184 gave a lower value (0.24) than that (0.28) of the whole amylose. Similarly, the λ_{max} values were in a longer wavelength range than those (526–531 nm) of whole amylopectins.

3.4. Gel-permeation chromatography of SAP

Saikai 194, Hoshiutaka, and Saikai 184 starches were selected as having characteristic properties, and their SAP structure was then examined in more detail. Saikai 194 and Saikai 184 starches had properties similar to japonica and indica rices, and Hoshiyutaka seemed to be an intermediate between them (Mizukami et al., 1996). Gel-permeation chromatography of the SAP (Fig. 2) showed a wide distribution of SAP molecules including a fair amount of small molecules which were generally difficult to detect. Saikai 194 showed two peaks, Hoshiyutaka had a peak with one or two shoulders and Saikai 184 were flat around the void volume, perhaps as a result of poor fractionation. The larger molecules showed lower β-AL than the smaller molecules, suggesting that the larger molecules were more highly branched.

3.5. The structure of the SAP subfractions

The SAP was fractionated into three subfractions of large (SAP-L), medium (SAP-M), and small (SAP-S) molecules, as indicated in Fig. 2, and their structures were subsequently analyzed (Table 5). Saikai 194 and Hoshiyutaka had similar proportions in all those subfractions and the SAP-L was the major component whereas the SAP-S (10%–14%) was the minor component. In Saikai 184, the SAP-M (43%) and SAP-S (36%) were the major components. Although SAP-L was the major fraction of Saikai 194 and Hoshiyutaka, it represented only a minor amount of the whole starch (2.5% and 2.4%, respectively), and in Saikai 184 it was only 0.9%.

The cultivar Saikai 194 had larger SAP-L ($\overline{DP}_n$, 790) molecules than the others (470, 490). However, all SAP-L fractions were similar in CL (17–18) and β-AL (54%–56%). These results suggested that all SAP-L fractions had branched structures similar to normally separated amylopectin although the molecular sizes were much smaller. The structures of the SAP-M and the SAP-S differed by cultivar as shown by the values of DP_n, CL, $\overline{NBL}$ and β-Al (Table 5). The CL of SAP-M of Saikai 194 and Saikai 184 were considerably longer than those of normal rice amylopectin, but that of Hoshiyutaka was in the normal range. The SAP-S had very small molecules having a DP_n of 23–34 and a $\overline{CL}$ of 17–23 and β-AL of 76%–85%. The $\overline{NBL}$ of the SAP-S were in the range of 0.4–0.5, showing that they were composed of linear and branched molecules. Judging from these results, the branched structures of SAP-L and SAP-M appeared to be similar to amylopectin, and this was confirmed by the following CL distributions. However, the SAP-S could not be regarded as amylopectin. Fig. 3 shows the CL distributions of SAP subfractions by size exclusion HPLC after debranching by isoamylase. The SAP-L and the SAP-M showed similar CL distribution patterns with two peaks (DP about 15 and 40) and one or two shoulders, as compared to the amylopectin. However, the CL distributions of the SAP-S were different from that of

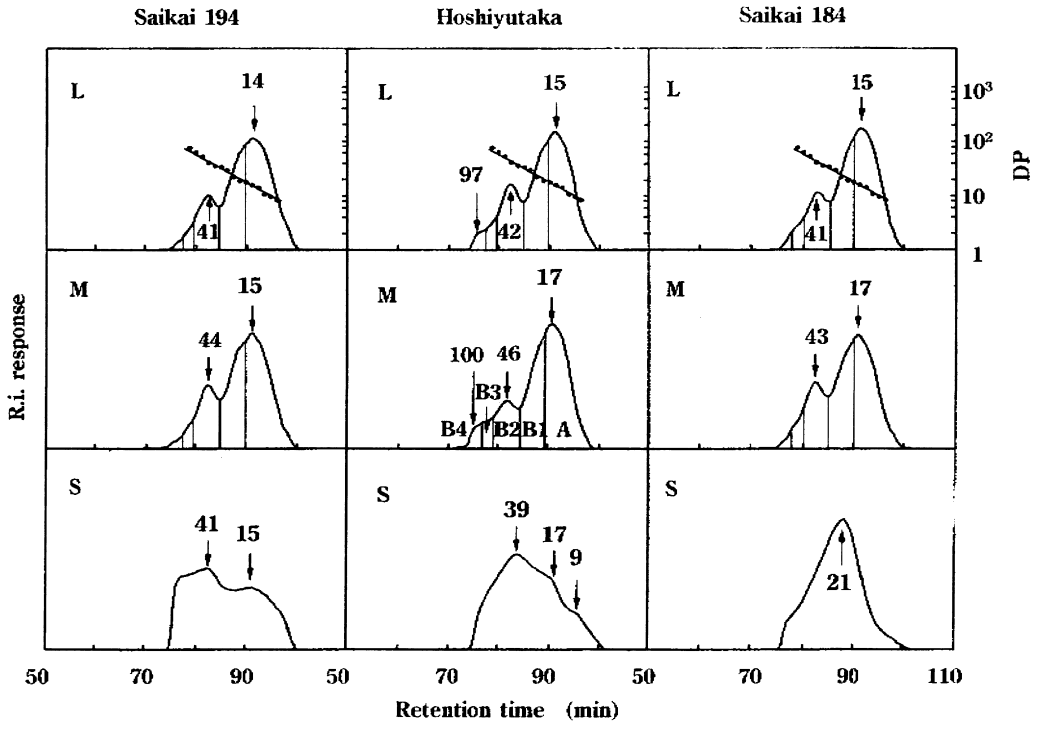

Fig. 3. Gel-permeation HPLC of the subfractions of SAP after isoamylolysis.(—), response of differential refractometer; (●), DP

the amylopectin, and the SAP-S seemed to be composed of one (Saikai 184), two (Saikai 194), and three (Hoshiyutaka) chain subfractions with different sizes. Saikai 194 had a larger amount of subfraction with a DP larger than 40, as compared to Hoshiyutaka, and Saikai 184, which contained almost none of the larger molecules.

The SAP-L and SAP-M chains were fractionated into five subfractions, the A and B1-4 is similar to Hizukuri (1986), in order of elution, as shown in Table 6, and were compared with those of the whole amylopectins. The CL distribution of SAP-L and that of the SAP-M were similar but differed slightly from amylopectin in the amounts of these chains. However, a significant difference was that they lacked extended long chains (LC1 and LC2) (Mizukami et al., 1996) which were found in rice amylopectins (Hizukuri et al., 1989; Takeda et al., 1987a). The SAP-M of Hoshiyutaka had larger amounts of B4 (4.4%) and of A (50.1%) and smaller amounts of B2 (16.0%) chains than those of both Saikai 194 (A; 46.8%, B2; 19.7%) and Saikai 184 (A: 42.5%, B2: 20.0%).

Table 6
Carbohydrate amounts (%) of the fractions of isoamylase-debranched SAP subfractions.

Cultivar	Subfraction	LC1	LC2	B4	B3	B2	B1	A
Saikai 194	L	nd[a]	-	1.4	3.1	16.7	28.0	50.8
	M	-	-	2.0	3.1	19.7	28.4	46.8
	AP[b]	0.2	0.3	0.7	4.7	20.6	26.9	46.6
Hoshiyutaka	L	-	3.2	4.0	19.8	27.5	45.5	
	M	-	-	4.4	4.4	16.0	25.1	50.1
	AP	1.1	2.6	3.8	4.6	19.3	24.7	43.9
Saikai 184	L	-	1.5	4.2	17.3	27.4	49.6	
	M	-	-	2.0	5.0	20.0	30.5	42.5
	AP	1.6	5.7	6.0	4.8	18.6	23.2	40.1

[a] nd – Not detectable
[b] Amylopectin from completely dispersed starch (Mizukami et al., 1996).

4. Discussion

In a previous study, we found that Hoshiyutaka and Saikai 184 starches were distinguishable from others in terms of structural and pasting properties, namely, Hoshiyutaka and Saikai 184 starches gave the lowest and highest maximum viscosities, and both with low breakdowns during posting, as was revealed by a rapid Visco Analyzer (Table 1). The $\overline{DP}_n$ and $\overline{DP}_w$ values of their whole amyloses were in a similar range to the others, but their $\overline{NBL}$ values were 4.5 and 3.5, which were considerably higher than those (1.0–1.5) of the others (Mizukami et al., 1996). The SAM of these two varieties were also characterized by having higher $\overline{DP}_n$, $\overline{DP}_w$; and $\overline{NBL}$ (2.5 and 2.0) that those of the others (0.6–0.8). The relatively high branching and consequently lower CL of the amylose molecules appears to be specific to Hoshiyutaka and Saikai 184, and it is this that may cause the solubilization of the larger amylose molecules.

It was reported that aqueous leaching is an excellent method of obtaining pure amylose (Banks & Greenwood, 1975) from potato and some cereal starches. However, the present results suggest that for the new rice cultivars, the

HWS was mainly SAP, of which the major components were small amylopectins. This implies that small amylopectin molecules dissolve more easily in hot water than large amylose molecules. However, it is necessary to confirm the present data using starches from other species. In addition, the amylopectins of these two cultivars (Hoshiyutaka and Saikai 184) had structures characterized by having larger amounts (3.7%–7.3%) of extended long chains than the others (up to 0.4%–1.2%), as was reported previously (Mizukami et al., 1996), while all the SAP molecules from the six rice cultivars were free from the extended long chains. This may suggest that the molecules having LC oppose solubilization possibly by complexing with lipids or anchoring deeply inside the crystalline domain and which subsequently withstands breakdown.

Analysis of the SAP subfraction showed that the SAP-L, and perhaps the SAP-M, were small amylopectin molecules, by their $\overline{DP}_n$, $\overline{CL}$, and β-AL values, and that SAP-S was a mixture of small amylose molecules and small fragments of amylopectin, or intermediate molecules. The significance of the presence of these molecules is not clear at present, and will be examined in future studies.

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