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STRUCTURES OF RICE AMYLOPECTINS WITH LOW AND HIGH AFFINITIES FOR IODINE

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ABSTRACT

The amylopectins from the apparent low- (japonica) and high-amylose (indica) varieties of rice showed, respectively, low (0.39–0.87 g/100 g) and high (1.62–2.57 g/100 g) affinities for iodine, average chain-lengths ($\overline{\text{c.l.}}$) of 19–20 and 21–22, and similar beta-amylolysis limits (56–59%). Gel filtration of the isoamylase-debranched amylopectins indicated that the amylopectins showing a high affinity for iodine contained lower proportions of short chains and higher proportions (up to 14.2%) of the long chains ($\overline{\text{c.l.}}$, 85–180). The long chains appeared to be derived from long B-chains with side chains widely spaced and located far from the non-reducing terminus. The apparent high-amylose (30–32%) rice starches had ordinary contents (15.5–18.5%) of amylose.

INTRODUCTION

Rice starches contain[1] 0–30% of amylose, and the content among varieties of rice varies with the ambient temperatures during development of the grain[1–6]. We found no significant differences in the structures of the amyloses from apparent low- and high-amylose starches[7], but the corresponding amylopectins showed low and high affinities, respectively, for iodine. Similar findings[1,8] have been reported, but the purities of the specimens were not examined. Some differences in the iodine-binding properties, chain lengths, and distributions of the amylopectins from various rice samples have been reported[4–6,9–12], but the experiments were carried out without pre-fractionation of the amylopectins from the rice starches.

We now report on the amylopectins from apparent low- (18–22%, three japonica varieties) and high-amylose (30–32%, three indica varieties)[13] rice starches.

EXPERIMENTAL

Materials. — The japonica and indica starches, other than Koshihikari starch,

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were the specimens described previously[7], and the Koshihikari starch was prepared by the same procedure as for the other japonica starches[7]. Beta-amylase was prepared[14] from sweet potatoes and recrystallised from aqueous ammonium sulfate in order to improve its stability on storage. Crystalline *Pseudomonas* isoamylase and *Klebsiella* pullulanase were obtained from Hayashibara Biochemical Laboratories Inc.

Preparation of rice amylopectin and its long-chain component. — Defatted rice starch was treated[7] with an aqueous mixture of 1-butanol and 3-methyl-1-butanol, kept at ~8° for 48 h, and then centrifuged (10,000*g*, 20 min, 4°). The supernatant solution was concentrated to ~30% volume at ~40°, and ethanol (2 vol.) and LiBr (25 mg) were added. The precipitated amylopectin was ground with ethanol, collected on a glass filter (G-2), washed with ethanol and ether, and dried *in vacuo* at room temperature over $CaCl_2$.

The long-chain fraction of an amylopectin was prepared as follows. The amylopectin was incubated with isoamylase (0.04 U/mg of amylopectin) at pH 3.5 and 45° for 2.5 h. The hydrolysis was complete after incubation for 1 h. To the solution was added 1-butanol (0.1 vol.), the mixture was kept at 30° for 2 h, the precipitated long-chain fraction was collected by centrifugation (3000 r.p.m., 20 min, room temperature), washed with aqueous 10% 1-butanol, and then dried *in vacuo* over $CaCl_2$.

Analytical methods. — Iodine affinities were determined by a modified amperometric titration procedure[15]. The amylopectin (100–120 mg) was dissolved in M KOH (5 mL), and then water (80 mL), M HCl (10 mL), and 0.4M KI (5 mL) were added successively. The solution was stirred at 25°, titrated continuously (~0.1 mL/min) with 1.67mM KIO_3 using a micro-tube pump (LKB Model 2132), and monitored by measurement of the electric current with Pt electrodes. The blue value was determined as described elsewhere[16]. The limiting viscosity number $[\eta]$ was determined at 22.5° in M KOH with an Ostwald viscometer. Carbohydrate was determined by the anthrone–sulfuric acid method[17]. The number-average d.p. values ($\overline{\text{d.p.}}_n$) of amylose and amylopectin were determined by the modified Park–Johnson method[18]. The method was originally developed for amylose, but was applicable to amylopectins using high concentrations (japonica amylopectins, ~10 mg/mL; indica amylopectins, ~15 mg/mL); the turbidity of the amylopectin gel was corrected for using an appropriate blank. The average chain-length ($\overline{\text{c.l.}}$) was determined by the rapid Smith-degradation method[18,19] and by hydrolysis with isoamylase[20]. Beta-amylolysis and the simultaneous degradation with beta-amylase and pullulanase were performed as described previously[18]. Phosphorus was determined[21] as inorganic phosphate after treatment with hot perchloric acid[22]. Phosphorus in D-glucose 6-phosphate residues was assayed by using D-glucose 6-phosphate dehydrogenase[23]. The distribution of the chain lengths of amylopectins was examined[11] by high-performance gel chromatography on three sequentially linked columns [TSK gel G3000SW and G2000SW (× 2), each 7.5 mm × 60 cm].

RESULTS AND DISCUSSION

The amylopectins from the apparent low- (japonica varieties) and high-amylose (indica varieties) rice starches showed a relatively wide range of affinities for iodine (Table I). Similar findings for normal rice starches have been reported[1,8] and the higher λ_{max} of the amylopectins of rice starches with higher apparent contents of amylose was indicated by gel filtration[12]. The indica amylopectins, especially IR42, showed high affinities for iodine, whereas the japonica amylopectins showed values similar to those of root, tuber, and other cereal amylopectins[16,24–26], although the Hokkaido amylopectin showed a slightly higher value. The blue value and λ_{max} of each rice amylopectin correlated with the affinity for iodine. The high affinity for iodine suggested contamination by amylose or the presence of amylopectins having long chains. The former possibility would require the IR42 amylopectin to contain ~13% of amylose. When the amylopectin was eluted from a column of Toyopearl HW-75F, a single peak (Fa) resulted (Fig. 1) together with a small retarded fraction (Fb). The other amylopectins showed similar elution profiles. Fb was the fraction in which rice amylose was eluted[7] and corresponded to ~10% of the amylopectin. The iodine-staining properties of Fb (blue value 0.249, λ_{max} 566 nm) differed from those of amylose, and were close to those of Fa (blue value 0.232, λ_{max} 564 nm) and the parent amylopectin. This finding implies that there was no contamination by amylose and suggests that the amylopectin had long chains.

TABLE I

PROPERTIES OF RICE AMYLOPECTINS

	Japonica			*Indica*		
	Hokkaido[a]	*Koshihikari*	*Sasanishiki*	*IR32*	*IR36*	*IR42*
Iodine affinity (g/100 g)	0.87	0.39	0.49	1.62	1.62	2.57
Blue value	0.077	0.049	0.051	0.150	0.156	0.232
λ_{max} (nm)	542	535	531	565	565	575
$[\eta]$ (mL/g)	137	137	134	150	170	165
$\overline{D.p._n}$[b]	11000	8200	12800	4700	5400	5800
Beta-amylolysis limit (%)	59	59	58	56	59	58
Chain length						
Smith degradation	19	20	19	21	21	22
Isoamylolysis	19	20	19	21	21	21
Organic phosphorus (p.p.m.)	8	11	13	22	29	11
Phosphorus at C-6 of the glucose residue (p.p.m.)	8	11	13	21	28	9

[a]Unknown variety, produced in Hokkaido. [b]Number-average degree of polymerisation.

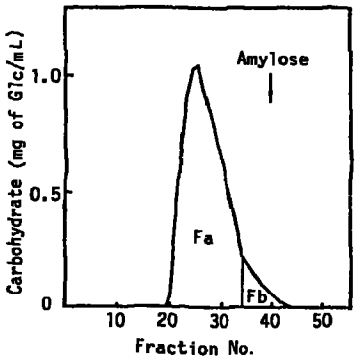


Fig. 1. Chromatography of IR42 amylopectin on Toyopearl HW-75F. The amylopectin (100 mg) suspended in ethanol (0.4 mL) was dissolved by the addition of M NaOH (1 mL). The solution was appropriately diluted with water, neutralised with 2M HCl, then made up to 10 mL, applied to a column (2.6 × 100 cm) of Toyopearl HW-75F, and eluted at 45° with 50mM NaCl (10-mL fractions).

The limiting viscosity numbes $[\eta]$ of the rice amylopectins (Table I) were lower than those of potato [182 (ref. 27) and 224 mL/g (ref. 20)] and sweet-potato (175–193 mL/g)[24] amylopectins, and similar to that of kuzu amylopectin (160 mL/g)[20]. The japonica amylopectins had lower $[\eta]$ values but higher $\overline{d.p.}_n$ values than the indica amylopectins, implying that the former may be more spherical in shape or may have a narrower distribution of molecular sizes. The beta-amylolysis limits of the rice amylopectins were similar to those of root and tuber amylopectins[17,20,24]. The average chain-lengths ($\overline{c.l.}$) of the rice amylopectins were $\overline{d.p.}$ 19–22 (*cf.* 17.0 for waxy-rice amylopectin[9], 23.7 for potato[20] and 23.6 for lily[16] amylopectin, and 21.1 for kuzu[20], 21.1 for tapioca[28], and 21–22 for sweet-potato[2] amylopectin). The $\overline{c.l.}$ values of the indica amylopectins were 1–2 greater than those of the japonica amylopectins. The rice amylopectins contained small proportions of 6-phosphate groups as reported previously[29], which were lower than those for the amylopectins from root and tuber starches[16,20,25,30].

Figure 2 shows the distributions of chain lengths of the rice amylopectins on gel-permeation h.p.l.c. after debranching with isoamylase. The properties of the three fractions (F1–F3) are summarised in Table II. All the amylopectins showed similar $\overline{c.l.}$ values for F2 and F3, and had similar proportions of F2, but the amylopectins (indica) with the high affinities for iodine contained lower proportions of F3 and higher proportions of F1 than the amylopectins (japonica) with low affinities for iodine. The presence of a fraction similar to F1, *i.e.*, a void-volume peak on Sephadex CL-2B chromatography which stained blue with iodine, has been reported for the amylopectins from apparent high-amylose rice starches[13], whereas h.p.l.c. indicated it to be almost absent[11] for waxy-rice amylopectin having a low affinity for iodine (0.07–0.09 g/100 g)[1,8]. Thus, the proportion of F1 correlated with the affinity of the amylopectin for iodine.

The fractions (Fppt) corresponding to F1 were obtained as a complex with

TABLE II

CARBOHYDRATE PROPORTIONS AND $\overline{d.p.}_w$[a] OF F1–F3 OF ISOAMYLASE-DEBRANCHED RICE AMYLOPECTINS

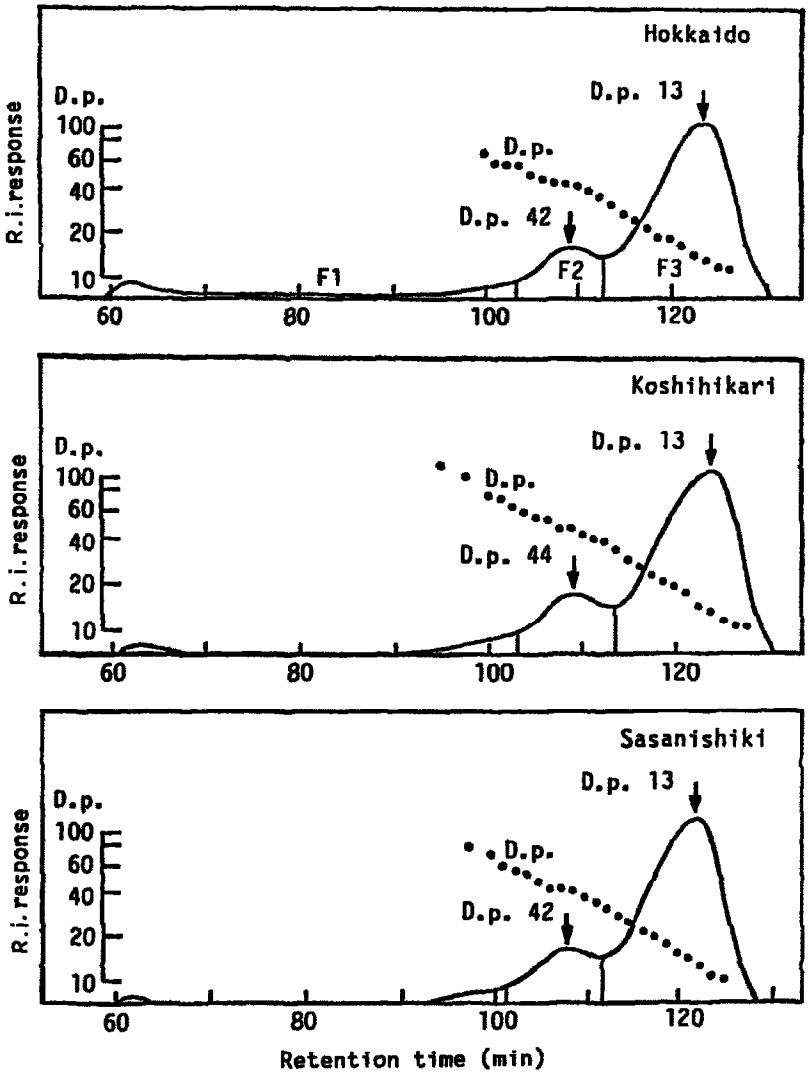
	Japonica			*Indica*		
	Hokkaido[a]	*Koshihikari*	*Sasanishiki*	*IR32*	*IR36*	*IR42*
Carbohydrate						
F1 (% of total)	9	6	7	14	15	20
F2 (% of total)	17	19	19	19	19	19
F3 (% of total)	74	75	74	67	66	61
F3/F2	4.3	3.9	3.9	3.5	3.5	3.2
Chain length ($\overline{d.p.}_w$)						
F2	41	44	41	44	43	42
F3	16	17	17	17	17	16

[a]Weight-average degree of polymerisation.

1-butanol from isoamylase-debranched amylopectins. The proportions of Fppt were similar to those of F1. Fppt showed (Table III) iodine-staining properties similar to those of amylose and had $\overline{d.p.}_n$ 120–220 and $\overline{c.l.}$ 85–180. These results indicated that Fppt contained long chains. However, the incomplete hydrolysis with beta-amylase and the $\overline{d.p.}/\overline{c.l.}$ ratios of >1 suggested that Fppt contained a small proportion of slightly branched molecules not debranched by isoamylase. The branches in these molecules appeared to be attached by α-(1→6) linkages since Fppt was degraded completely by treatment simultaneously with beta-amylase and pullulanase. Similar branches have been observed in rice[7] and some other amyloses[18,24,31].

The chains stretching across some clusters in the amylopectin molecule, as suggested by Hizukuri[11], according to the cluster model[32,33], seem to be relatively long. However, the chains were considered not to contribute to the affinity of the amylopectins for iodine because of the low affinity of waxy-rice amylopectin for iodine, in which these chains probably have closely spaced side-chains. On the other hand, the long chains (F1 or Fppt) derived from the non-waxy-rice amylopectins appeared to originate from long B-chains with side chains widely spaced and located far from the terminal glucosyl group. Alternatively, there might be unusually long A-chains, but no direct evidence was obtained. The presence of long B-chains was supported by the fact that the beta-limit dextrins, obtained from amylopectins with high affinities for iodine, had low or relatively high affinities for iodine (Table IV). The IR36 amylopectin, of which the beta-limit dextrin had a low affinity for iodine, may contain a higher proportion of long B-chains with side chains located far from the terminal glucosyl group. The long chains may be distributed uniformly in the amylopectin molecules since attempts at fractional precipitation with iodine, cyclohexanol, or pinacol failed.

Thus, the amylopectins showing high affinities for iodine appear to contain



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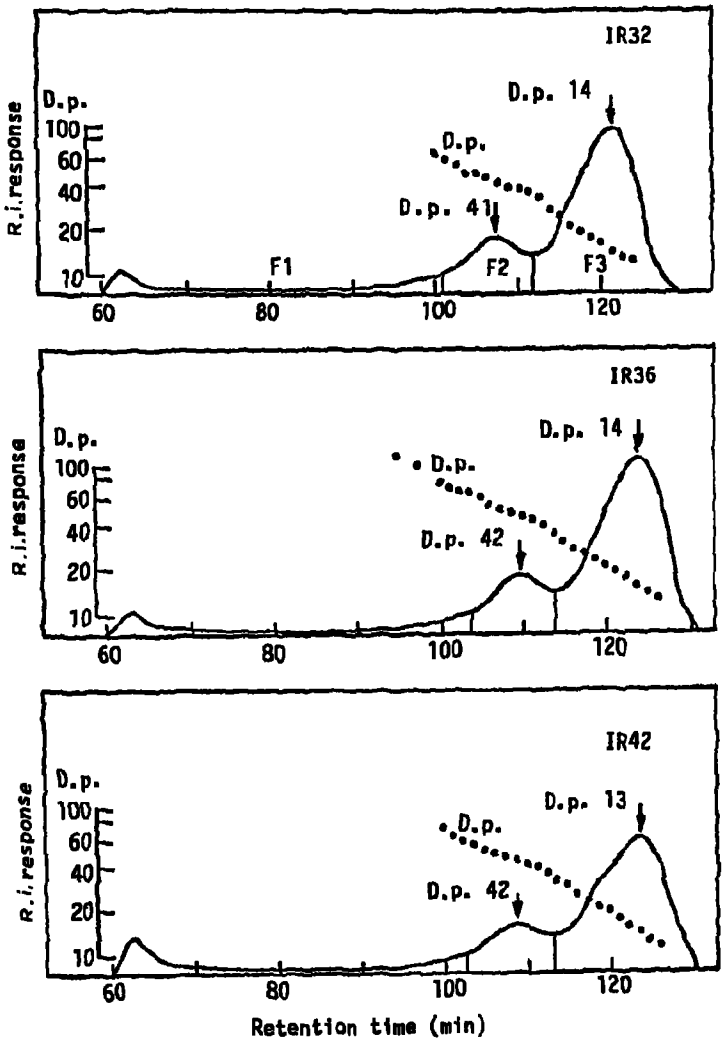

Fig. 2. Gel-filtration h.p.l.c. of rice amylopectins debranched with isoamylase. Each amylopectin was incubated[10] with isoamylase, the enzyme was inactivated, the mixture was filtered[10], and an aliquot (1 mL, 10 mg) was injected into the chromatograph. Elution was carried out at 0.53 mL/min with 0.1M sodium phosphate buffer (pH 6.1) containing 0.02% of sodium azide at a flow rate of 0.53 mL/min with detection using a differential refractometer (——). D.p. (●) was determined as described previously[10].

TABLE III

PROPERTIES OF Fppt (LONG-CHAIN FRACTIONS) OBTAINED BY PRECIPITATION OF ISOAMYLASE-DEBRANCHED RICE AMYLOPECTINS WITH 1-BUTANOL

	Japonica			*Indica*		
	Hokkaido[a]	*Koshihikari*	*Sasanishiki*	*IR32*	*IR36*	*IR42*
Yield from amylopectin (LCF) (%)	7.9	4.6	5.0	11.5	11.7	14.2
LCF/F1[a]	0.88	0.77	0.71	0.82	0.78	0.71
Iodine affinity (g/100 g)	19.0	17.3	17.4	15.9	14.4	15.7
Blue value	1.31	1.25	1.24	1.18	1.14	1.23
λ_{max} (nm)	629	624	623	620	620	623
$\overline{D.p.}_n$	220	150	180	120	120	140
Chain length	180	120	130	110	85	130
Beta-amylolysis limit (%)	79	85	83	86	82	83
Beta-amylolysis limit with pullulanase (%)	101	99	97	101	100	99

[a]See Table II.

TABLE IV

IODINE AFFINITIES OF BETA-LIMIT DEXTRINS[a] FROM RICE AMYLOPECTINS

	Japonica			*Indica*		
	Hokkaido[a]	*Koshihikari*	*Sasanishiki*	*IR32*	*IR36*	*IR42*
Iodine affinity (g/100 g)	0.66	0.22	0.33	0.86	0.17	0.83

[a]Determined with the beta-amylolysate of the amylopectins after the removal of coagulated protein.

higher proportions of the long B-chains and lower proportions of the short chains than the amylopectins showing low affinities for iodine. The proportions of these chains in the indica amylopectins appear to result in higher [η] values but similar beta-amylolysis limits (Table I) compared with the japonica amylopectins.

The carbohydrate contents of the fractions corresponding to F2 and F3 varied among the rice amylopectins with the variety[4–6,10,11]. However, these studies were performed using isoamylase-debranched, or debranched and then fractionated, starches. Therefore, the long-chain fraction (F1) was eluted together with the amylose fraction on gel filtration or removed as a complex with 1-butanol before gel filtration. The present study confirmed the presence of the long-chain fraction, *i.e.*, long B-chains with side chains widely spaced and located far from the terminal glucosyl group, in various amounts in rice amylopectins and the almost complete absence of these chains in waxy-rice amylopectin, as previously reported[11]. Examination of the tendency of retrogradation of the amylopectins containing higher

amounts of these chains is of interest since amylopectins with longer chain-lengths have been suggested to undergo rapid retrogradation[28]. This and previous[7] studies suggest that the starches from different varieties of rices and, perhaps, from rices grown under different ripening temperatures differ mainly in the structure of the amylopectin rather than that of the amylose.

The apparent content of amylose is calculated from the affinities of starch and amylose for iodine, assuming that that of amylose is 18–20 g/100 g, and various apparent contents (15–32%) of amylose for normal rice starches have been reported[1,4,13]. The present study suggests that the actual contents of amylose of some of these starches are considerably lower because of the relatively wide range (0.37–2.74 g/100 g)[1,8] of the iodine affinities of their amylopectins. Table V shows the contents of amylose for the japonica and indica rice starches in relation to the affinities of their amylopectins for iodine. The actual contents of amylose for the indica rice starches were much lower than their apparent contents, and similar to those for the japonica rice starches.

TABLE V

AMYLOSE CONTENTS OF RICE STARCHES BASED ON IODINE AFFINITIES OF STARCH AND ITS FRACTIONS

	Iodine affinity (g/100 g)			*Amylose content (%)*	
	Amylose[a]	*Amylopectin*	*Starch*[b]		
Japonica					
Hokkaido	20.3	0.87	4.36	18.0[c]	(21.8)[d]
Koshihikari	20.1[e]	0.39	3.69	16.7	(18.5)
Sasanishiki	20.6	0.49	4.00	17.5	(20.0)
Indica					
IR32	20.3	1.62	5.08	18.5	(25.4)
IR36	21.1	1.62	4.94	17.0	(24.7)
IR42	20.0	2.57	5.27	15.5	(26.4)

[a]From ref. 7. [b]Defatted by repeated dissolution in hot dimethyl sulfoxide followed by precipitation with ethanol. [c]Calculated with the following equation (i.a., iodine affinity): $(i.a._{starch} - i.a._{amylopectin})/(i.a._{amylose} - i.a._{amylopectin}) \times 100$. [d]Apparent content, calculated with the following equation: $i.a._{starch}/i.a._{amylose}\ (20.0) \times 100$. [e]Prepared by the method described previously[7] but without ultracentrifugation for the purification.

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