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# STRUCTURES OF INDICA RICE STARCHES (IR48 AND IR64) HAVING INTERMEDIATE AFFINITIES FOR IODINE

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ABSTRACT

The amyloses of rice starches (IR48 and IR64) having intermediate affinities for iodine (i.a.) had number- and weight-average d.p. values of 930 and 1200, and 3300 and 3420, respectively, and average chain-lengths ($\overline{c.l.}$) of 380 and 450. They comprised branched (~6 chains on average) and unbranched molecules in the molar ratios 1:2 and 1:3, respectively, and the former appeared to be larger. The amylopectins had i.a. of 0.63 and 0.87 g/100 g and $\overline{c.l.}$ of 20 and 21, respectively, and were composed of long, medium, and short chains in the weight ratios 9:21:70. The amylose contents were 17.0 and 18.3%, respectively. The normal rice starches contained similar amounts of amyloses with similar structures, regardless of their i.a., but the amylopectin from the higher-i.a. starch had a higher i.a. and a larger proportion of long chains.

## INTRODUCTION

Starches from various varieties of rice differ[1] in iodine affinities (i.a.), and this was considered to be due to the different contents of amylose. However, the starches with low and high i.a. contain similar amounts of amyloses, but the amylopectins have different structures[2–4]. The amylopectins from the high-i.a. starches have large proportions of long chains and long average chain-lengths ($\overline{c.l.}$). We now report on the structures and contents of the amyloses of rice starches having intermediate i.a., namely, indica IR48 and IR64.

## EXPERIMENTAL

*Materials.* — Indica rice starches IR48 (final gelatinisation temperatures, 64.5°) and IR64 (70.5°) were prepared[5] from milled flour by exhaustive extraction

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of the protein with sodium dodecyl benzenesulfonate. Each starch was fractionated into amylose and amylopectin by the method of Takeda *et al.*[2]. The yields of the amyloses from 10 g (dry weight) of the IR48 and IR64 starches were 1.6 and 1.5 g, respectively, and those of the amylopectins were 7.2 and 7.3 g (dry weight), respectively. The beta-limit dextrins ($\beta$-LD) from the amyloses were also prepared[6]. Sweet-potato beta-amylase[7] was recrystallised from aqueous ammonium sulfate. The long-chain fraction of an amylopectin was prepared[3] by precipitation with 1-butanol from its isoamylolysate. Crystalline *Pseudomonas* isoamylase and Toyopearl HW-75F were obtained from Hayashibara Biochemical Laboratories Inc. (Okayama) and Tosoh Co., Ltd. (Tokyo), respectively.

*Analytical methods.* — The purity of the amyloses was examined[8] by chromatography on Toyopearl HW-75F. The i.a. was determined at 25° by a modified[3] amperometric titration[9]. The blue value[10] and limiting viscosity number $[\eta]$ (M KOH, 22.5°)[11] were determined as described. The number-average d.p. ($\overline{\text{d.p.}}_n$) of the amyloses and amylopectins were determined by the modified Park–Johnson method[12]. The $\overline{\text{c.l.}}$ of the amyloses was determined by the rapid Smith-degradation method[12] with minor modifications[8]. The average number of chains per molecule ($\overline{\text{n.c.}}$) was calculated as $\overline{\text{d.p.}}_n/\overline{\text{c.l.}}$ The weight-average d.p. ($\overline{\text{d.p.}}_w$) and distribution of d.p. of the amyloses were determined[13] by gel-permeation h.p.l.c., using connected columns (Tosoh TSKgel G6000PW, G4000PW, and G3000PW) with a differential refractometer (Tosoh RI-8000) and a low-angle laser-light-scattering photometer (Tosoh LS-8) as detectors. The isoamylolysis of amylose was carried out as described[12]. The $\overline{\text{c.l.}}$ of the amylopectins was determined by the rapid Smith-degradation method[14] and by hydrolysis with isoamylase[11]. The chain-length distribution of the amylopectins was examined[15] by gel-permeation h.p.l.c., using connected columns (Tosoh, TSKgel G3000SW and G2000SW × 2) with the detectors noted above. Carbohydrate was determined by the phenol–sulfuric acid method[16]. Phosphorus was determined[17] as inorganic phosphorus after treatment with hot perchloric acid[18]. The content of 6-phosphate was assayed using D-glucose 6-phosphate dehydrogenase[19].

## RESULTS AND DISCUSSION

*Structure of amylose.* — Gel-permeation chromatograms on Toyopearl HW-75F (Fig. 1) indicated that the amyloses from indica starches IR48 and IR64 were free from amylopectin, since they gave a single peak and there was no carbohydrate in the void volume[8]. Table I indicates that the amyloses had the same i.a., blue value, and $\lambda_{max}$, suggestive of similar structures. Their $[\eta]$ values were higher than those (180–216 mL/g) of rice amyloses from low- and high-i.a. starches, whereas their $\overline{\text{d.p.}}_n$ and $\overline{\text{d.p.}}_w$ were similar or slightly higher than those of other rice amyloses[2].

The amyloses had similar elution profiles on gel-permeation h.p.l.c. and gave single peaks with maxima at a similar d.p. (Fig. 1). Their profiles resembled those

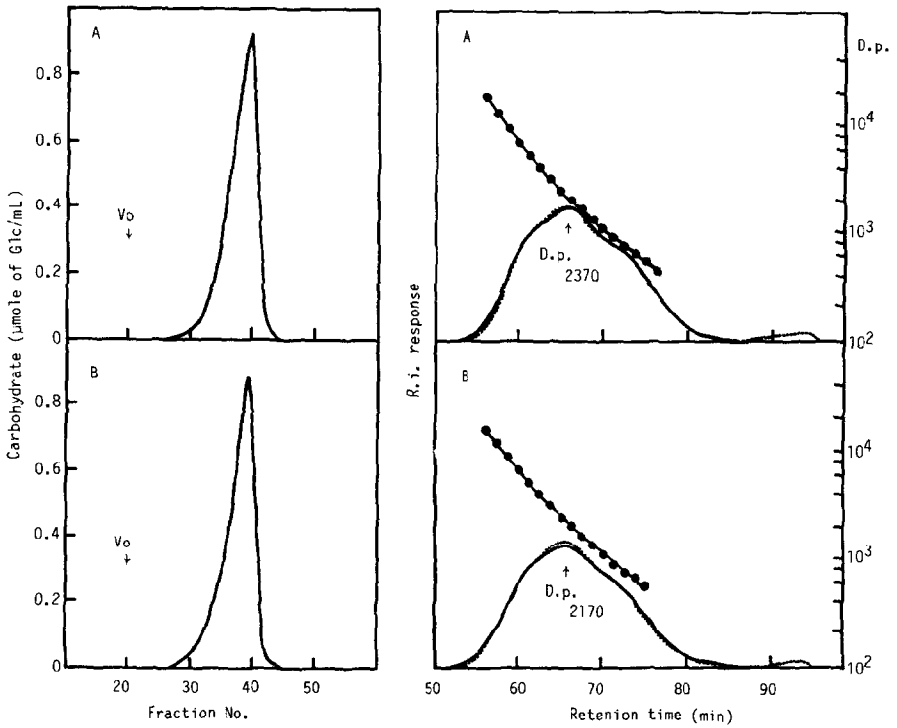


Fig. 1. Gel-permeation patterns of the IR48 (A) and IR64 (B) amyloses and their isoamylolysates on Toyopearl HW-75F (left), and connected columns of TSKgel G6000PW, G4000PW, and G3000PW with h.p.l.c. (right). The conditions were as previously described[13]: — and ···, response of the differential refractometer (R.i.) for amylose and the isoamylolysate, respectively; ●, D.p.

of the low- and high-i.a. amyloses, but they had slightly wider distributions of d.p. The slightly concave curves of d.p. on the chromatogram suggest the larger molecules to be more spherical in shape than the smaller molecules, due to a higher degree of branching (see below). Their $\overline{c.l.}$ and $\overline{n.c.}$ were close to those of amylose from IR36, a high-i.a. starch, but slightly different from those ($\overline{c.l.}$, 230–320; 3.5–4.3 chains) of other rice amyloses. They gave high beta-amylolysis limits (β-a.l.) and were almost free from phosphorus (<1 p.p.m.), as are other rice amyloses[2].

The properties of β-LDs derived from the branched molecules of the amyloses were similar (Table I). The β-LDs showed i.a., iodine-staining properties, and $\overline{d.p.}_n$ and $\overline{d.p.}_w$ values similar to those of the parent amyloses and for other rice amyloses[2,4]. Their $\overline{d.p.}_n$ values resembled those of the low- and high-i.a. β-LDs, but their $\overline{d.p.}_w$ values were a little higher (2480–3500). Their $\overline{c.l.}$s were within the range 90–155 for low- and high-i.a. β-LDs. Their $\overline{n.c.}$ indicated that the branched molecules had ~6 chains on average, similar to that from IR36 but a little lower than those (7.5–9.7 chains) from other varieties of rice. The molar fractions of branched and unbranched molecules indicated the unbranched molecules to be abundant in the amyloses, as for other rice amyloses (molar fractions, 0.57–0.68)

TABLE I

PROPERTIES OF RICE AMYLOSES AND THEIR BETA-LIMIT DEXTRINS ($\beta$-LD)

| | *IR48* | | *IR64* | |
|---|---|---|---|---|
| | *Amylose* | *$\beta$-LD* | *Amylose* | *$\beta$-LD* |
| Iodine affinity (g/100 g) | 20.9 | 19.2 | 20.9 | 19.2 |
| Blue value | 1.44 | 1.32 | 1.45 | 1.32 |
| $\lambda_{max}$ (nm) | 656 | 650 | 653 | 654 |
| $[\eta]$ (mL/g)[a] | 243 | | 249 | |
| $\overline{D.p.}_n$[b] | 930 | 700 | 1020 | 840 |
| $\overline{D.p.}_w$[c] | 3420 | 3960 | 3300 | 4260 |
| $\overline{D.p.}_w/\overline{d.p.}_n$ | 3.7 | 5.7 | 3.2 | 5.1 |
| Apparent d.p. distribution[d] | 440–14,000 | 420–13,000 | 480–12,000 | 710–16,000 |
| Average chain-length ($\overline{c.l.}$) | 380 | 125 | 450 | 140 |
| Average number of chains ($\overline{n.c.}$) | 2.5 | 5.7 | 2.3 | 6.1 |
| Beta-amylolysis limit ($\beta$-a.l.) (%) | 82 | | 87 | |
| Molar fractions[e] of | | | | |
| branched molecule | 0.32 | | 0.25 | |
| unbranched molecule | 0.68 | | 0.75 | |
| Isoamylolysate | | | | |
| $\overline{D.p.}_n$ | 480 | | 590 | |
| $\overline{N.c.}$ | 1.3 | | 1.3 | |
| $\beta$-A.l. | 90 | | 95 | |

[a]Limiting viscosity number. [b,c]Number and weight-average d.p. [d]$D.p._w$ values of the sub-fractions (10% amylose by weight) having the lowest and highest molecular weights. [e]Calculated[7] from the numbers of chains of amylose and its $\beta$-LD.

except for IR32, a high-i.a. amylose (0.51). The gel-permeation chromatograms of the $\beta$-LDs (Fig. 2) showed single peaks with maxima at d.p. 2820 and 3710, and the IR64 $\beta$-LD had abundant large molecules. Their profiles were similar to those of the parent amyloses. These findings and the similarity in molecular sizes of the $\beta$-LDs and their parent amyloses imply that the branched molecules were larger than the unbranched molecules. Thus, the intermediate-i.a. amyloses had structures similar to that of the IR36 amylose rather than to those of rice amyloses, but all rice amyloses, so far examined[2,4], have similar structures in comparison with those from other sources[6].

The chromatogram (Fig. 2) in gel-permeation h.p.l.c. showed that isoamylolysis of the branched molecule caused no significant change in hydrodynamic volume of the amylose, but produced a small amount of short chains. The chains, isolated[3] in yields of ~1.4% (by weight) from each isoamylolysate by removal of the large molecules as complexes with 1-butanol, had $\overline{d.p.}_n$ 16 and 18, which are close to the $\overline{c.l.}$ of rice amylopectins. The incomplete beta-amylolysis and $\overline{n.c.}$ of >1 of debranched amyloses (Table I) indicated incomplete hydrolysis with isoamylase, as reported for other rice amyloses[2], perhaps due to the extremely long side-chains. Using the equation[12] $[(\overline{d.p.}_{n\ \text{amylose}}/\overline{d.p.}_{n\ \text{debranched amylose}} - 1)/(\overline{n.c.}_{\text{amylose}} - 1)] \times 100$, the debranching was calculated to be 61% for IR48, and 55% for IR64,

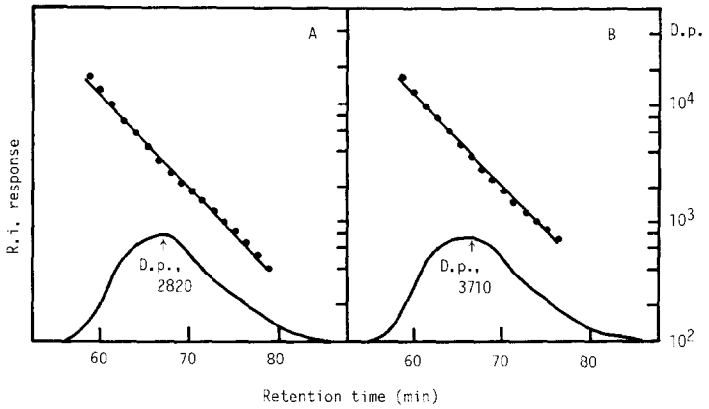


Fig. 2. Gel-permeation h.p.l.c. of the beta-limit dextrins from the IR48 (A) and IR64 (B) amyloses on connected columns of TSKgel G6000PW, G4000PW, and G3000PW. The conditions were as previously reported[13]: —, response of the differential refractometer (R.i.); ●, D.p.

implying that ~3 in 5 branch linkages of the branched molecule were hydrolysed. Thus, the average branched amylose from the rice comprises three extremely long and three short chains with a distribution of d.p. similar to that of amylopectin.

*Structure of amylopectin.* — Table II shows that the intermediate-i.a. amylopectins have similar structures. Their i.a. values were intermediate of those (0.39–0.49 g/100 g) of the low-i.a. amylopectins, except for the Hokkaido amylopectin (0.87 g/100 g) and the high-i.a. amylopectins (1.62–2.57 g/100 g). Their blue values and $\lambda_{max}$ values correlated well with the i.a. values. The $[\eta]$ values of the amylopectins were higher than those of the low-i.a. amylopectins, and similar to, or lower than, those of the high-i.a. amylopectins, and the $\overline{d.p.}_n$ values were similar to those of the low-i.a. amylopectins but considerably higher than those of the high-i.a. amylopectins ($\overline{d.p.}_n$ 4700–5800). The $\beta$-a.l. values of the rice amylopectins were similar. The $\overline{c.l.}$ of the amylopectins appeared to be intermediate of

TABLE II

PROPERTIES OF RICE AMYLOPECTINS

| | *IR48* | *IR64* |
|---|---|---|
| Iodine affinity (g/100 g) | 0.86 | 0.63 |
| Blue value | 0.10 | 0.08 |
| $\lambda_{max}$ (nm) | 548 | 542 |
| $[\eta]$ (mL/g) | 157 | 152 |
| $\overline{D.p.}_n$ | 9,000 | 15,000 |
| $\overline{C.l.}$ | | |
| Smith degradation | 20 | 21 |
| Isoamylolysis | 20 | 20 |
| $\beta$-A.l. (%) | 58 | 59 |
| Total phosphorus (p.p.m.) | 5 | 14 |
| 6-Phosphate as phosphorus (p.p.m.) | 5 | 12 |

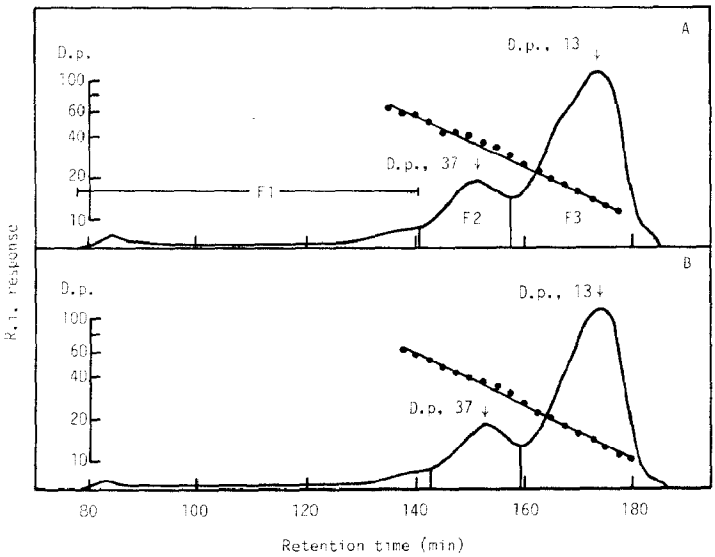


Fig. 3. Gel-filtration h.p.l.c. of the IR48 (A) and IR64 (B) amylopectins debranched with isoamylase. Each debranched amylopectin was prepared and analysed as previously reported[15]: —, response of the differential refractometer (R.i.); ●, D.p.

those of the low- (19–20) and high-i.a. (21–22) amylopectins. They contained trace amounts of 6-phosphate groups, as reported for other rice amylopectins[2].

The amylopectins had similar chain-length distributions (Fig. 3) and proportions of long- (F1), medium- (F2), and short-chain (F3) fractions, with similar $\overline{d.p.}_w$ (Table III). The proportion of F1 was the same as that of the Hokkaido amylopectin and intermediate of those of other low- (6–7%) and high-i.a. (14–25%) amylopectins, implying that the amylopectin of higher i.a. had a higher proportion of F1. The proportion of F2 of the intermediate-i.a. amylopectins was slightly higher than those (19%) of the low- and high-i.a. ones. However, the proportion

TABLE III

CARBOHYDRATE PROPORTIONS AND $\overline{D.P.}_w$ OF F1–F3 FOR ISOAMYLASE-DEBRANCHED RICE AMYLOPECTINS

| | *IR48* | *IR64* |
|---|---|---|
| *Carbohydrate (% of total)* | | |
| F1 | 9 | 9 |
| F2 | 21 | 21 |
| F3 | 70 | 70 |
| F3/F2 | 3.3 | 3.3 |
| $\overline{C.l.(d.p._w)}$ | | |
| F2 | 42 | 40 |
| F3 | 16 | 16 |

TABLE IV

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

| | *IR48* | *IR64* |
|---|---|---|
| Yield of LCF from amylopectin (%) | 7.1 | 7.9 |
| Iodine affinity (g/100 g) | 16.2 | 17.0 |
| Blue value | 1.28 | 1.26 |
| $\lambda_{max}$ (nm) | 617 | 615 |
| $\overline{D.p._n}$ | 150 | 140 |
| $\overline{C.l.}$ | 140 | 130 |
| $\beta$-A.l. (%) | 94 | 95 |

of F3 was intermediate of those of the low- (74–75%) and high-i.a. (61–67%) amylopectins. On the other hand, F2 and F3 of all rice amylopectins had similar $\overline{d.p._w}$ values[2].

The long-chain fractions (LCF) of the amylopectins were obtained (Table IV) as complexes with 1-butanol from the isoamylolysates, and their yields were similar to that of the Hokkaido amylopectin and intermediate of those of other low- (4.6–5.0%) and high-i.a. (11.5–14.2%) amylopectins[2]. The blue value and $\lambda_{max}$ of LCFs were similar to those of rice amyloses. Their $\overline{d.p._n}$ and $\overline{c.l.}$ were intermediate of those ($\overline{d.p._n}$ 120–220, $\overline{c.l.}$ 85–180) of the low- and high-i.a. LCFs. The incomplete hydrolysis with beta-amylase and the value of $\overline{d.p._n}/\overline{c.l.}$ of >1 for the LCFs implied that small amounts of branch linkages were not hydrolysed with isoamylase, similar to the low- and high-i.a. amylopectins. These findings indicated the presence of long chains in the intermediate-i.a. amylopectins, as in other rice amylopectins. The long chains appeared to originate from long B-chains with widely spaced side-chains, because of relatively high i.a. values of the amylopectins and their beta-limit dextrins (0.27–0.37 g/100 g). These long chains with side chains may be similar to poorly branched amylose. Thus, the amylopectin of higher i.a. has a higher proportion of long chains but a lower proportion of short chains.

*Iodine affinity of starch and its amylose content.* — The i.a. values of the intermediate-i.a. starches were intermediate of those of the low- (3.69–4.00 g/100 g) and high-i.a. (4.94–5.27 g/100 g) starches[2]. Hokkaido starch was regarded

TABLE V

IODINE AFFINITIES OF IR48 AND IR64 STARCHES AND THEIR CONTENTS OF AMYLOSE

| *Starch* | *Iodine affinity[a] (g/100 g)* | *Amylose content[b] (%)* |
|---|---|---|
| IR48 | 4.36 | 17.0 (21.8)[c] |
| IR64 | 4.33 | 18.3 (21.6)[c] |

[a]Specimens were defatted[2] by repeated dissolution in hot dimethyl sulfoxide and precipitation with ethanol. [b]Calculated using the equation[10] (i.a., iodine affinity): $(i.a._{starch} - i.a._{amylopectin})/(i.a._{amylose} - i.a._{amylopectin}) \times 100$. [c]Apparent content, calculated using the equation: $[i.a._{starch}/i.a._{amylose}\ (20)] \times 100$.

previously[2] as a low-i.a. starch, but, judging from its i.a. (4.36 g/100 g) and structure, it is now regarded as an intermediate-i.a. starch.

The apparent contents of amylose for the intermediate-i.a. starches were calculated as 21.6 and 21.8% from their starch and assumed amylose i.a. (20 g/100 g) without consideration of the amylopectin i.a. (Table V). However, the actual contents of amylose, taking into account the i.a. of the respective amylose and amylopectin[10], were 17.3 and 18.3%, which resembled those (15.5–18.5%, 17.7% on average) of the low- and high-i.a. starches[2], indicating that the low-, intermediate-, and high-i.a. starches have similar contents of amylose.

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