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Structures of amylose subfractions with different molecular sizes

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ABSTRACT

The structures of subfractions (30–38% of the total), having $\overline{\text{d.p.}}_n$ ~ 2500, ~ 1500, and ~ 400 ($\overline{\text{d.p.}}_w$ ~ 6300, ~ 1800, and ~ 600), of amyloses from maize and rice have been analysed and found to be similar. The subfractions with $\overline{\text{d.p.}}_n$ ~ 400 preponderated (66%) and were rich in linear molecules (maize 71%, rice 75%), whereas those with $\overline{\text{d.p.}}_n$ ~ 2500 contained mainly branched molecules (maize 66%, rice 61%). The branched molecules in the subfractions with $\overline{\text{d.p.}}_n$ ~ 2500 and ~ 400 had large (maize 14.5, rice 10.5) and small (maize 3.1, rice 2.6) numbers of chains on average, respectively, and that in the subfraction with $\overline{\text{d.p.}}_n$ ~ 1500 had an intermediate number. The branched molecules in each subfraction comprised the short-chain fraction (probably $\overline{\text{d.p.}}_n$ ~ 18), and the larger branched molecules had a larger number of the short chains. The C chain of the larger branched molecules appeared to be the longer.

INTRODUCTION

Amyloses isolated[1,2] from starches of various plants have characteristic molecular size and distribution thereof, degree of branching, and molar fraction of branched molecules[1,3–11]. The structure of the branched molecules, which was elucidated by analysis of a beta-limit dextrin because no method was available for its quantitative separation from the linear molecules, also depends on the origin[3–10]. Details of the structure of amylose have been revealed by investigation of the subfractions. Thus, subfractions of the amylose of maize, which were soluble and insoluble in aqueous 1-butanol, had different molar fractions and structures of the branched molecules[11]. Amylose from the water chestnut contains three components with various sizes and different structures[8]. We now report on three subfractions of amyloses from maize and rice obtained by gel-permeation chromatography.

EXPERIMENTAL

Materials. — Amyloses were isolated from defatted[1] starches of maize (a white dent variety from South Africa)[4] and rice (Indica IR48)[7] by the method of Lansky *et al.*[12] and were purified[1] by ultracentrifugation and repeated recrystallisation from aq. 10%

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1-butanol. The amyloses were free from amylopectin[2] and had iodine affinities of 20.1 g/100 g for maize and 20.9 g/100 g for rice. Crystalline *Pseudomonas* isoamylase was the product of Hayashibara Biochemical Laboratories Inc. (Okayama). Sweet-potato beta-amylase[13] was recrystallised from aq. ammonium sulfate. Toyopearl HW-65F and HW-60F were obtained from Tosoh Co. Ltd. (Tokyo). Other reagents of the highest grade were from Wako Pure Chemical Industries, Ltd. (Tokyo).

Fractionation of amylose. — The amylose solution (10 mg/mL, 15 mL), prepared as described[14], was eluted from columns (2.6 × 100 cm) of Toyopearl HW-65F and HW-60F in series, at 45°, with water at 30 mL/h, and fractions (15 mL) were collected. Three subfractions were obtained and each was lyophilised.

Analytical methods. — The blue value[15] and the number-average degree of polymerisation ($\overline{\mathrm{d.p.}}_{n}$)[14] were determined as described. The weight-average d.p. ($\overline{\mathrm{d.p.}}_{w}$) was determined by h.p.l.c. gel-permeation chromatography on columns of TSK-gel G6000PW, G4000PW, and G3000PW (Tosoh) in series, using a differential refractometer (Erma, ERC 7152) and a low-angle laser-light-scattering photometer[16] (Tosoh, LS-8) as detectors. The beta-amylolysis limit ($\beta_{\mathrm{a.l.}}$) was determined as described[2]. The average chain length ($\overline{\mathrm{c.l.}}$) was determined by the Smith-degradation method[14] with minor modifications[2]. The average number of chains per molecule ($\overline{\mathrm{n.c.}}$) was calculated as $\overline{\mathrm{d.p.}}_{n}/\overline{\mathrm{c.l.}}$. The molar fraction of branched molecules in each subfraction was determined by the tritium-labelling method[17]. Carbohydrate was determined by the phenol–H_2SO_4 method[18]. The treatment with isoamylase was carried out as described[14].

RESULTS AND DISCUSSION

The amyloses of maize ($\overline{\mathrm{d.p.}}_{n}$ 930, $\overline{\mathrm{d.p.}}_{w}$ 2410)[4] and rice ($\overline{\mathrm{d.p.}}_{n}$ 930, $\overline{\mathrm{d.p.}}_{w}$ 3420)[7] each gave a single unsymmetrical peak on gel-permeation chromatography[4,7] (Fig. 1), which was divided into three subfractions (F1/3) having large, medium, and small molecular sizes, respectively (Fig. 1). Each subfraction then gave a symmetrical peak (Fig. 2) and the d.p. values at the top of each peak were, respectively, 5630, 1820, and 570 for maize, and 6140, 1840, and 610 for rice. For each whole amylose, a plot of d.p. against retention time gave a slightly concave slope but, for the subfractions, the slopes were different,

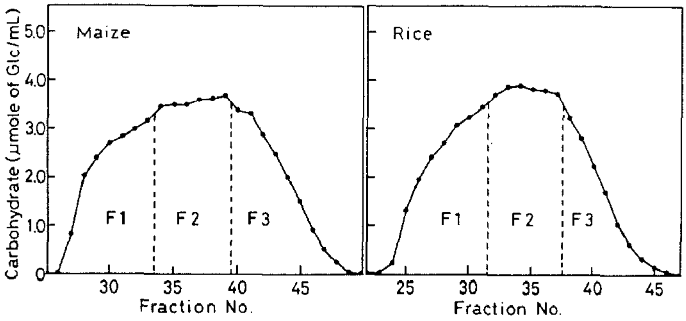

Fig. 1. Gel-permeation chromatograms of maize and rice amyloses on Toyopearl HW-65F and HW-60F.

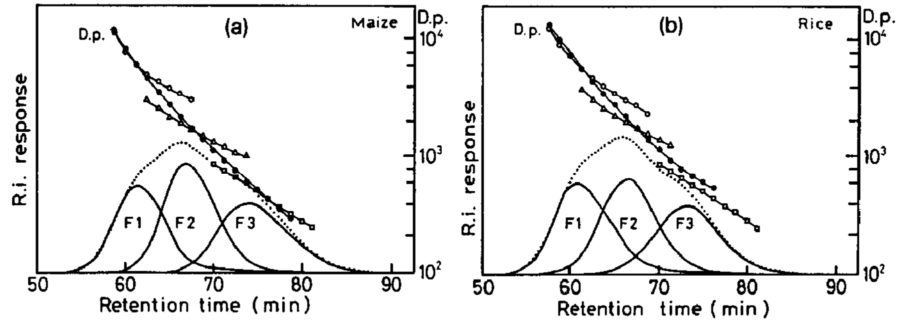

Fig. 2. Gel-permeation h.p.l.c. of subfractions (—) of amylose (···) from (a) maize and (b) rice on columns of TSK-gel G6000PW, G4000PW, and G3000PW in series; d.p.: ●, amylose; o, F1; △, F2; □, F3.

suggesting differences in molecular shape.

The properties of each group of the subfractions listed in Table I were similar. The weight proportions were similar (30–38%), but F1 comprised the lowest molar proportion (maize 9%, rice 14%) and F3 the highest (maize and rice 66%). Thus, small molecules preponderated in each amylose. The subfractions except maize F3 had similar blue values and λ_{max}. F1 had the highest $\overline{\text{d.p.}}_n$ (maize 2720, rice 2230) and $\overline{\text{d.p.}}_w$ (6410, 6280) values and F3 the lowest (maize $\overline{\text{d.p.}}_n$ 390, $\overline{\text{d.p.}}_w$ 570; rice 410, 680). The molecular sizes of F1/3 roughly resembled those of the corresponding subfractions ($\overline{\text{d.p.}}_n$ 2060, 1270, 390; $\overline{\text{d.p.}}_w$ 9260, 2230, 550) of water chestnut amylose[8]. F1 showed a higher ratio of $\overline{\text{d.p.}}_w/\overline{\text{d.p.}}_n$ (maize 2.36, rice 2.82) than F2 and F3 (1.16–1.66), suggesting that F1 had the wider d.p. distribution.

F1 and F3 showed similar average chain-lengths ($\overline{\text{c.l.}}$, 250, 330), lower than those

TABLE I

Properties of amylose subfractions from maize and rice

Subfraction	*Maize*			*Rice*		
	F1	*F2*	*F3*	*F1*	*F2*	*F3*
Proportion (% of total)						
Weight	31	38	31	34	36	30
Mole[a]	9	25	66	14	20	66
Blue value	1.37	1.39	1.14	1.40	1.37	1.34
λ_{max} (nm)	649	642	614	652	645	626
Number-average d.p. ($\overline{\text{d.p.}}_n$)	2720	1280	390	2230	1670	410
Weight-average d.p. ($\overline{\text{d.p.}}_w$)	6410	1740	570	6280	1930	680
$\overline{\text{D.p.}}_w/\overline{\text{d.p.}}_n$	2.36	1.36	1.46	2.82	1.16	1.66
Average chain length ($\overline{\text{c.l.}}$)	275	435	250	330	520	295
Average number of chains ($\overline{\text{n.c.}}$)	9.9	2.9	1.6	6.8	2.5	1.4
Beta-amylolysis limit ($\beta_{a.l.}$, %)	75	89	90	81	89	92

[a] Calculated from the proportion by weight and the $\overline{\text{d.p.}}_n$.

(435–520) for F2. The average number of chains ($\overline{\text{n.c.}}$, maize 1.6, rice 1.4) of F3 was lower than those (2.9, 2.5) of F2, and much lower than those (9.9, 6.8) of F1. Maize F1 showed a higher $\overline{\text{n.c.}}$ than rice F1, owing to a higher molar fraction of branched molecules and a higher degree of branching (see below). The $\overline{\text{n.c.}}$ values of F1 were higher than that (2.9) of the large subfraction from water chestnut[8], whereas F2 and F3 had $\overline{\text{n.c.}}$ values similar to those of its medium (2.1) and small (1.3) subfractions, respectively. These findings suggest that maize and rice amyloses are rich in large branched molecules compared with water chestnut amylose, since the former had higher molar fractions (maize[4] 0.44, rice[7] 0.32) of branched molecules than the latter[8] (0.11). The subfraction having a larger $\overline{\text{n.c.}}$ gave a lower beta-amylolysis limit ($\beta_{\text{a.l.}}$). The molar fraction of branched molecules in each subfraction was determined by the tritium-labelling method[17] (Table II). F1 showed the highest molar fraction (maize 0.66, rice 0.61), whereas F3 showed the lowest (0.29, 0.25), indicating that the preponderant molecules in F1 and F3 were branched and linear molecules, respectively, as suggested earlier[11].

The $\overline{\text{n.c.}}$ of the branched molecules in each subfraction was calculated from the molar fractions of branched (MF_B) and linear (MF_L) molecules and the $\overline{\text{n.c.}}$ of the subfraction ($\overline{\text{n.c.}}_{\text{SUB}}$) by the equation: $\overline{\text{n.c.}}$ of branched molecule = $(\overline{\text{n.c.}}_{\text{SUB}} - MF_L)/MF_B$. The $\overline{\text{n.c.}}$ values of the branched molecules of F1/3 were 14.5, 6.6, and 3.1 for maize and 10.5, 5.2, and 2.6 for rice, respectively, indicating the large branched molecules to be highly branched. This inference is in keeping with the finding that the large subfraction gave a steep slope for a plot of d.p. against retention time (Fig. 2). There were more heavily branched molecules ($\overline{\text{n.c.}}$ 20) in the maize subfraction ($\overline{\text{d.p.}}_n$ 580, $\overline{\text{d.p.}}_w$ 5910, $\overline{\text{n.c.}}$ 4.1, MF_B 0.16), which was obtained as a soluble fraction on incubation in aqueous 1-butanol[11], probably due to branched molecules with few branches being insoluble.

The isoamylolysates of F1 and F3 showed a lower $\overline{\text{d.p.}}_n$ (320–395) than those (maize 605, rice 645) of F2. The isoamylolysis reduced largely the $\overline{\text{d.p.}}_n$ of F1 and slightly its $\overline{\text{d.p.}}_w$ (Table III), due to the production of a relatively large number of short chains (see below). All the isoamylolysates gave $\overline{\text{n.c.}}$ values of > 1, and were incompletely degraded with beta-amylase ($\beta_{\text{a.l.}}$, 82–98%), indicating the presence of resistant linkages.

TABLE II

Molar fractions of linear and branched molecules, and average number of chains ($\overline{\text{n.c.}}$)[a] of branched molecules

Subfraction	*Maize*			*Rice*		
	F1	*F2*	*F3*	*F1*	*F2*	*F3*
Molar fraction						
Linear molecules (MF_L)	0.34	0.66	0.71	0.39	0.64	0.75
Branched molecules (MF_B)	0.66	0.34	0.29	0.61	0.36	0.25
$\overline{\text{N.c.}}$ of branched molecules	14.5	6.6	3.1	10.5	5.2	2.6

[a] $\overline{\text{N.c.}} = (\overline{\text{n.c.}}_{\text{subfraction}} - MF_L)/MF_B$.

TABLE III

Properties of isoamylolysates and beta-limit dextrins of amylose subfractions

Subfraction	*Maize*			*Rice*		
	F1	*F2*	*F3*	*F1*	*F2*	*F3*
Isoamylolysate						
$\overline{\text{D.p.}}_n$	335	605	320	395	645	330
$\overline{\text{D.p.}}_n/\overline{\text{d.p.}}_n$ (native)	0.12	0.47	0.82	0.18	0.39	0.80
$\overline{\text{D.p.}}_w$	4620	1630	540	4960	1820	600
$\overline{\text{D.p.}}_w/\overline{\text{d.p.}}_w$ (native)	0.72	0.94	0.95	0.79	0.94	0.88
$\overline{\text{N.c.}}$	1.2	1.4	1.3	1.2	1.2	1.1
$\beta_{\text{a.l.}}$ (%)	82	91	94	90	93	98
Weight proportion of short-chain fraction (%)	7	2	1	5	1	2
Beta-limit dextrin						
$\overline{\text{D.p.}}_w$	5090	1510	500	5120	1820	520
$\overline{\text{D.p.}}_w/\overline{\text{d.p.}}_w$ (native)	0.79	0.87	0.88	0.82	0.94	0.76

Such linkages seem to be α-(1→6) because simultaneous hydroysis with pullulanase and beta-amylase degraded the amyloses completely into maltose[1,14,19], and they might be in close proximity as found in amylopectin[20,21].

The isoamylolysis slightly shifted the gel-permeation profiles of the subfractions towards smaller molecular sizes, and produced a small proportion of a short-chain fraction (SCF) (Fig. 3). The slight shifts suggest that the C chain (*i.e.*, the chain having the reducing terminal) of the branched molecules may be fairly long and probably similar to the size of each subfraction. Maize and rice F1 gave SCF values of 7 and 5%, respectively (*c.f.* 1–2% for F2 and F3). SCF originated from amylose and not from an amylopectin impurity. The amyloses were shown to be free of amylopectin by the high affinity for iodine (maize[4] 20.1 g/100 g and rice[7] 20.9 g/100 g) and by the chromatograms[4,7] on Toyopearl HW-75F where amylopectin was eluted at the exclusion volume, whereas amylose was totally included[2], and even 0.5% of amylopectin impurity was clearly detectable. The high affinity of their beta-limit dextrins for iodine (maize[4] 19.1 g/100 g, and rice[7] 19.2 g/100 g) also indicated the amyloses to be pure. The SCF was too small to allow determination of its molecular size by h.p.l.c.[16], but it appeared to have a short side chain with $\overline{\text{d.p.}}_n \sim 18$, which was obtained from isoamylolysates of rice[7] amylose and the subfraction of maize amylose[11]. The number of short chains ($\overline{\text{n.c.}}_{SC}$) of the branched molecules could be calculated from the $\overline{\text{d.p.}}_n$ ($\overline{\text{d.p.}}_{nSUB}$) and MF_B of the subfraction and a proportion (%) of SCF (P_{SCF}), assuming that the $\overline{\text{d.p.}}_n$ of SCF was 18 by the equation:

$$\overline{\text{N.c.}}_{SC} \text{ of branched molecule} = [(\overline{\text{d.p.}}_{nSUB}) / P_{SCF}/18/MF_B] \times 100$$

The $\overline{\text{n.c.}}_{SC}$ values of F1/3 were 16, 4.2, and 0.7 for maize, and 10, 2.6, and 1.8 for rice,

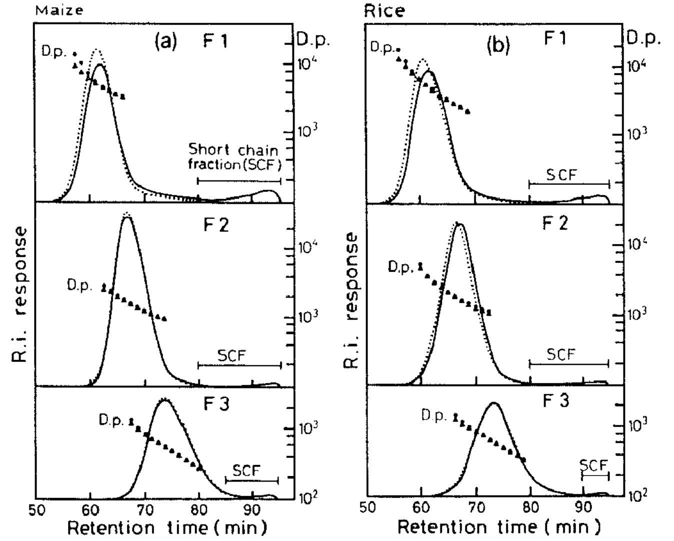

Fig. 3. Gel-permeation h.p.l.c. of isoamylolysates (—; ▲, d.p.) of the subfractions (···; ●, d.p.) of (a) maize and (b) rice on columns of TSK-gel G6000PW, G4000PW, and G3000PW in series.

respectively. The values were close to the $\overline{\text{n.c.}}$ of the respective branched molecules (Table II), suggesting that the main side chain of each subfraction was short and that the large branched molecules had a large number of the short chains. These results accord with the finding that F1 reduced the $\overline{\text{d.p.}}_n$ largely, but the $\overline{\text{d.p.}}_w$ only slightly, on isoamylolysis (see Table III).

Maize F1 appears to comprise branched molecules with long side chains of d.p. probably 200–2000, since isoamylolysis increased the elution of carbohydrate between the main and SCF peaks (Fig. 3). A similar long side chain was shown by the chromatogram of a tritiated side chain[17] and was suggested to be in a maize subfraction obtained as a soluble fraction by incubating the amylose at 40° with 1-butanol[11]. For rice F1, a long side chain may be present because a small proportion of carbohydrate was produced between the peaks by isoamylolysis (Fig. 3). These results imply that the large branched molecules have long side chains. The mechanism of synthesis of such long side chains is of interest because its direct transfer by branching enzyme is unknown.

The elution profile of a beta-limit dextrin (β-LD) of each subfraction was similar to that of the parent subfraction, but slightly moved towards the side of small molecules (Fig. 4), as described for amyloses[10,11]. The β-LD from each subfraction showed a $\overline{\text{d.p.}}_w$ which was a little lower than that of the present subfraction (Table III). The production of large and small β-LDs from respective large and small branched molecules suggests that branch linkages were located randomly on the chains.

Thus, the branched molecules of the amyloses of maize and rice differ in size and preponderate in the fraction with large $\overline{\text{d.p.}}_n$.

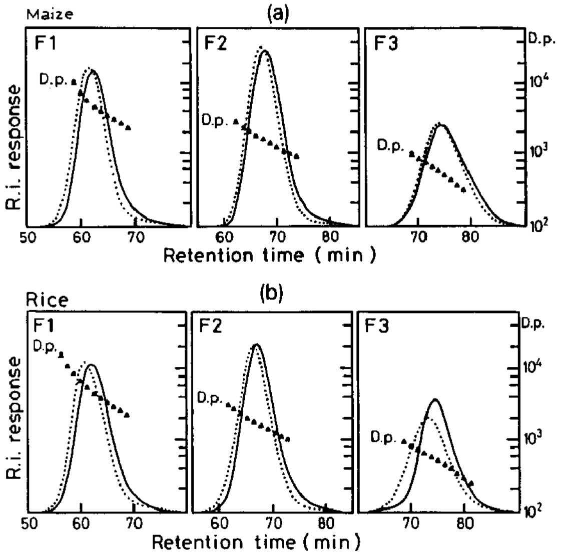

Fig. 4. Gel-permeation h.p.l.c. of beta-limit dextrins (—; ▲, d.p.) of the subfractions (···; ●, d.p.) of (a) maize and (b) rice on columns of TSK-gel G6000PW, G4000PW, and G3000PW in series.

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