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Structures of branched and linear molecules of rice amylose

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ABSTRACT

The structures of branched and linear molecules of the isolated amylose (number-average dp 850) from rice were examined by the tritium labeling method and gel-permeation chromatography. The branched and linear molecules showed similar, symmetrical distributions on a molar basis with a dp of 710 and 540 at the peak, respectively, and different distributions on a weight basis with a dp of 4440 and 710 at the peak, respectively. The branched and linear molecules had a $\overline{dp}_n$ of 1180 and 740, respectively, and the ratios of the branched to the linear molecule were 0.22:0.78 by mole and 0.32:0.68 by weight. The chromatogram of the reduced tritiated isoamylolyzate, from the reduced amylose, revealed that the branched molecule had side chains with various sizes (dp ~ 10–4000). The side chains had a peak dp of 21. The long side chain accompanied another chain(s). Thus, the branched molecule is a structural intermediate between true linear amylose and amylopectin.

INTRODUCTION

The branched molecule in isolated amyloses[1,2] has been suggested to be larger than the linear molecule (true amylose)[3–9] and to be an intermediate component between true amylose and amylopectin[10,11]. However, the structures of the branched and linear molecules have been estimated by analyzing the amylose, its beta-limit dextrin[3–9], and subfractions[11,12], as to the degree of polymerization, beta-amylolysis limit, number of chains per molecule, and molar fraction of the branched molecule, since no method is available for their quantitative separation. Tritium labeling[13] of the reducing terminal of amylose by reduction with sodium [^{3}H]borohydride enables the determination of the reducing terminal, the molar fraction of the branched molecule, and the molar distribution of amylose molecules in gel-permeation chromatography. We now report an application of this method to the analysis of the structures of the branched and linear molecules of rice amylose.

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EXPERIMENTAL

Materials.—The amylose [number-average dp ($\overline{dp}_n$) 850, determined by colorimetric methods[14]] was fractionated from defatted rice (japonica, Nihonbare) starch by the method of Lansky et al.[1] and purified[2] by ultracentrifugation and repeated recrystallization from aq 10% 1-butanol. The specimen was free from amylopectin, judging from the gel-permeation chromatogram[15] and the value (21.6 g/100 g) of iodine affinity. The reduced and tritiated amylose and the reduced amylose were prepared as previously described[13]. Sodium [^{3}H]borohydride (18.1 GBq/mmol) was obtained from Daiichi Chemical Industries Co., Ltd. (Tokyo). Toyopearl HW-65F, 60F, and 55S were products of Tosoh Co. Ltd. (Tokyo). Bio-Gel P-2 (−400) and crystalline *Pseudomonas* isoamylase were obtained from Bio-Rad Laboratories Inc. and Hayashibara Biochemical Laboratories Inc. (Okayama), respectively. Sweet-potato beta-amylase[16] was recrystallized from aqueous ammonium sulfate.

Gel-permeation chromatography and analytical methods.—The solution (1 mL, 30 mg) of the reduced and tritiated samples[13] was applied to a column (2.2 × 75 cm) having two layers (of equal volume). The top layer was composed of a mixture of Toyopearl HW-65F, 60F, and 55S (1:1:1 by vol) and the bottom layer of Bio-Gel P-2. The column was kept at 45°C and eluted downwards with 0.1 M phosphate buffer (pH 6.2) at 2 mL/h. Fractions of 2 mL were collected. An aliquot (1 mL) of each fraction was transferred to a glass-fiber filter (Whatman GC-50, 35 × 35 mm) and the radioactivity was determined[13] after drying. The molar concentration (reducing terminal, nmol/mL) of molecules of each fraction (R_F) is given by $(cpm_F/\Sigma cpm_F) \cdot \Sigma C_F \cdot 1000/(\overline{dp}_n$ of amylose, 850), where C_F and cpm_F are the carbohydrate concentration (μmol as glucose equiv/mL) and cpm of each fraction, respectively. The dp of each fraction (dp_F) was calculated as C_F/R_F.

The molar fraction of the branched molecule of each fraction (MF_{FB}) was determined by the method of Takeda et al.[13] with minor modifications. An aliquot (0.4 mL) of the fraction was incubated at 37°C for 3 h with beta-amylase solution (16 μL, 625 U/mL of M acetate buffer, pH 4.8) or M acetate buffer, pH 4.8 (16 μL). The solutions (0.3 mL) were placed on a glass-fiber filter, and the radioactivities before (*b*) and after (*a*) beta-amylolysis were determined after washing the filters. MF_{FB} was calculated as a/b. Carbohydrate was determined by the phenol–H_2SO_4 method[17].

RESULTS AND DISCUSSION

Fig. 1 shows the molar fraction of the branched molecule of each fraction (MF_{FB}), dp_F, and distributions of the rice amylose ($\overline{dp}_n$ 850) on molar and weight bases by gel-permeation chromatography of the reduced and tritiated amylose. The chromatogram indicated a single elution profile with a peak dp of 540 and 1260 on

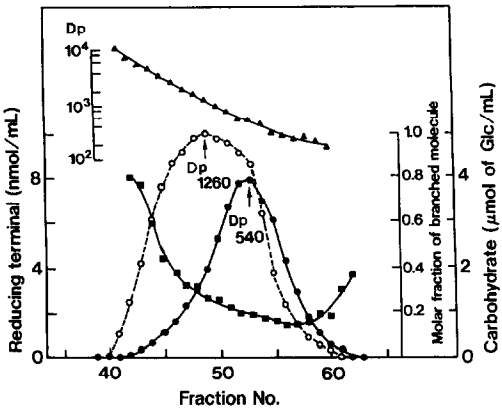

Fig. 1. Gel-permeation chromatogram of the reduced and tritiated amylose of rice (see Experimental): ●, reducing terminal (R_F); ○, carbohydrate (C_F); ■, molar fraction of the branched molecule (MF_{FB}); ▲, dp (dp_F).

molar and weight bases, respectively. These values were smaller than those (710 and 1580) of maize amylose ($\overline{dp}_n$ 930)[13]. The apparent dp_n distribution (the subfractions, 10% by mol, of the largest and smallest molecules) was 220–3200. MF_{FB} decreased from 0.80 to 0.15 with increase of the fraction number up to 58 and then increased to ~ 0.40. These results indicate that large amylose molecules are rich in branched molecules, whereas medium and small amylose molecules are rich in linear molecules, similar to the case of the rice (IR48) amylose subfractions[12] with different sizes; very small amylose molecules contain a rather greater amount of the branched molecule.

The concentrations of the reducing terminal (nmol/mL) of the branched (R_{FB}) and linear (R_{FL}) molecules of each fraction are given by $R_F \cdot MF_{FB}$ and $R_F \cdot (1 - MF_{FB})$, respectively, where R_F is for whole molecules of each fraction. The carbohydrate concentrations (μmol as glucose equiv/mL) of the branched (C_{FB}) and linear (C_{FL}) molecules are given by $R_{FB} \cdot dp_F/1000$ and $R_{FL} \cdot dp_F/1000$, respectively. The plots of R_{FB} and R_{FL} against the fraction number gave distributions of the branched and linear molecules on a molar basis, respectively, whereas those from C_{FL} and C_{FB} are on a weight basis (Fig. 2). The branched and linear molecules had a similar, symmetrical distribution with a peak dp of 710 and 540 on a molar basis, respectively, and a different distribution with a peak dp of 4440 and 710 on a weight basis. The branched molecule (dp_n 200–4900) was distributed more widely than the linear molecule (220–2400).

The $\overline{dp}_n$ of the branched and linear molecules given by $\Sigma C_{FB}/\Sigma R_{FB}$ and $\Sigma C_{FL}/\Sigma R_{FL}$ were 1180 and 740, respectively, indicating that the branched molecule was larger than the linear molecule on average, as suggested for other amyloses[3,4,6–10]. The values for the branched and linear molecules might be a little smaller and larger, respectively, than the actual values because a branched molecule having the same dp_n as a linear molecule has a smaller hydrodynamic volume, due

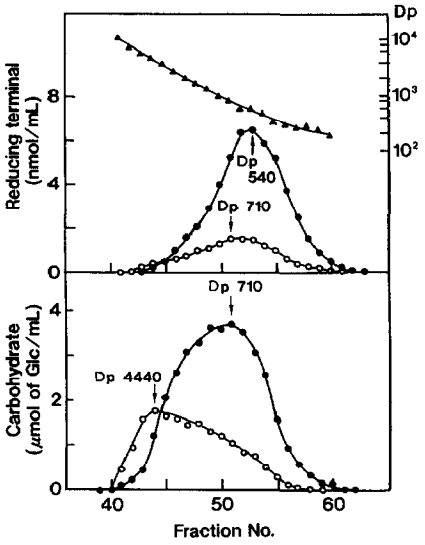

Fig. 2. Distributions of the branched (○; R_{FB}, C_{FB}) and linear (●; R_{FL}, C_{FL}) molecules on molar (top) and weight (bottom) bases.

to branching[5]. However, these values were similar to those (1130–1660 and 920–1110, respectively) of other rice amyloses ($\overline{dp}_n$ 920–1110), calculated[10] from the $\overline{dp}_n$ of amylose and its beta-limit dextrin (β-LD), and its MF_B and MF_L assuming the beta-amylolysis limit ($\beta_{a.l.}$) of the branched molecule to be 39%. The $\overline{dp}_n$ ratio of the linear to the branched molecule was 1:1.6, being in the range 1:1.3–2.1 of other rice amyloses[10].

The molar ratio of the branched to the linear molecule, given by $\Sigma R_{FB}/\Sigma R_F : \Sigma R_{FL}/\Sigma R_F$, was 0.22:0.78, resembling that (0.25:0.75) obtained from the number of chains of the amylose and its beta-limit dextrin (β-LD)[3], and the weight ratio, $\Sigma C_{FB}/\Sigma C_F : \Sigma C_{FL}/\Sigma C_F$, was 0.32:0.68. From the weight ratio and the $\beta_{a.l.}$ (80%) of the amylose, the $\beta_{a.l.}$ of the branched molecule may be estimated to be 38%, resembling that (39%) mentioned above.

Fig. 3 shows the distribution of tritiated molecules in the isoamylolyzate from the reduced and tritiated amylose. The molecules involved the C chain (the chain having the reducing terminal) from the branched molecule, but the chromatogram could not distinguish it from tritiated linear molecules and branched molecules with isoamylase-resistant linkages[14]. However, a similar distribution to the parent amylose suggests that the C chain has a $\overline{dp}_n$ resembling that of the parent amylose. Similar observations have been reported for rice and maize amyloses[9,12,13].

The [^{3}H]borohydride reduction of the isoamylolyzate from the reduced amylose labels only side chains of the branched molecule. The previous gel-permeation chromatography on a mixture of Toyopearl HW-65F and 60F[13] was suitable only for the analysis of long labeled chains because of the lack of separation of short labeled chains from a radioactive impurity in the [^{3}H]borohydride reagent. How-

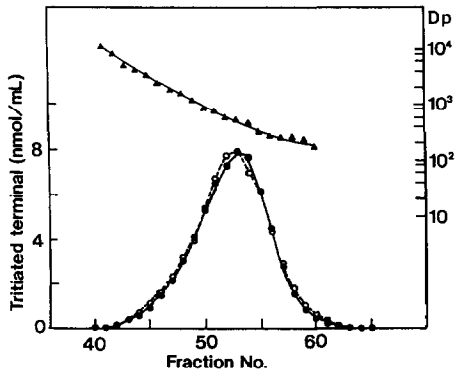

Fig. 3. Gel-permeation chromatogram of the tritium-labeled chains (●) of the isoamylolyzate from the reduced tritiated amylose of rice. For reference, the chromatogram (○) and dp (▲) of the reduced tritiated amylose (Fig. 1) are included.

ever, an additional layer of Bio-Gel P-2 revealed a molar distribution of whole labeled chains from the branched molecule (Fig. 4). The chains were distributed fairly widely (dp ~ 10–4000) and the peak dp was 21. The dp of the largest chain was similar to that (~ 5000) of maize amylose[13].

Large labeled chains were separated into two fractions, F1 and F2 in order of the elution (Fig. 4), and concentrated by lyophilization to determine their MF_B because long chains contain some isoamylase-resistant linkages[11]. The MF_B values for F1 and F2 were 0.84 and 0.38, respectively, indicating that they both contained branch linkages and longer chains, and that F1 obtained a higher proportion of branched chains. The linkages were α-(1 → 6) because simultaneous incubation with pullulanase and beta-amylase completely degraded the amylose into maltose. These lines of evidence indicate that the branched molecule contains the long side chain accompanying another chain(s) (B chain), supporting the model[11] for the

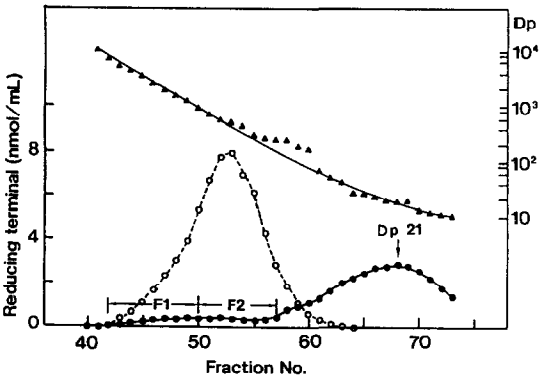

Fig. 4. Gel-permeation chromatogram of the labeled side-chains (●) released by isoamylase from the reduced amylose of rice. For reference, the chromatogram (○) and dp (▲) of the reduced tritiated amylose of rice (Fig. 1) are included.

large branched molecules from maize. On the other hand, both F1 and F2 also contained linear chains (MF_L 0.16 and 0.62, respectively). The short side chains ($\overline{dp}_n$ ~ 21) were preponderant in the branched molecule (Fig. 4). This is similar to the case of other rice amyloses ($\overline{dp}_n$ 16–19)[9]. These results imply that the branched molecule is composed of side chains with dp ~ 10–4000. The molar ratios of the very long (dp > 200), long (30–200), and short (10–30) side chains were 0.8 : 0.6 : 4. The long side chain may be produced by branching enzyme since the maize enzymes can transfer[18] chains with a dp of > 200. The present results indicate that the branched molecule has a structure intermediate between true linear amylose and amylopectin.

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